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THE UNIVERSITY OF ALBERTA

STOMACH MOVEMENTS AND DIGESTA TRANSIT IN RUMINANTS

by

ANDRE LIRETTE

(C)

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

IN

ANIMAL BIOCHEMISTRY

DEPARTMENT OF ANIMAL SCIENCE

EDMONTON, ALBERTA

SPRING 1987

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled STOMACH MOVEMENTS AND DIGESTA TRANSIT IN RUMINANTS submitted by ANDRE LIRETTE in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY in ANIMAL BIOCHEMISTRY.

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ABSTRACT

Five experiments were undertaken to clarify digesta and stomach movements in ruminants. In the first experiment, a quantitative schematic rumino-reticulum model was developed to describe the kinetics of forage particles in the digestive tract using grass stems mordanted with Cr at very low concentrations. Particle kinetics in four fistulated steers, fed with brome grass hay, were studied using a Latin square design. The ratio of the mass of the large particle pool (>3.35 mm) to the small particle pool (<3.35 mm) in the rumino-reticulum was 2:1. Major amounts of volatile fatty acids (VFA), CO_2 and CH_4 were produced from the large particle pool. A substantial portion (20 $\pm$ 11%) of the hay was rapidly solubilized. Material leaving the rumino-reticulum as large particles, small particles and soluble components were equivalent to 5, 25 and 0-20 % of feed dry matter (DM). The size of the particles influenced their turnover time in the post-ruminal tract. In the second experiment the possibility of a relationship between the respiration cycle and timing of ruminant stomach contractions was investigated using four ruminally cannulated steers and four sheep fitted with rumen and abomasal cannulae. A high degree of coincidence between inspiratory movements and the

second biphasic contraction of the reticulum was found which may favour passage of digesta to the omasum. In the third experiment, a new endoscopic technique using a modified fibre-optic endoscope was proven to be useful in the study of rate of passage, movement, and breakdown of particulate matter. In the fourth experiment the interrelationships between buoyancy and chemical composition of bromegrass hay, rumen and feces particles were studied. It was concluded that there was an interrelationship between buoyancy, physical attributes, shape, size, chemical and nutritive composition of different types of particles. In the last experiment, the effects of diet, and stress (acute cold or acute psychological stress) on forestomach motility in cattle were studied. Diets did not modify the contraction frequencies of the forestomachs. Acute cold and psychological stress produced significant (20 to 100%) increases in the frequency of forestomach contractions.

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I. INTRODUCTION

Clearance of feed residues from the rumino-reticulum is a major process in determining the intake and nutritive value of forages. Clearance depends on two interacting processes; breakdown (physical reduction in particle size plus microbial digestion), and physical removal from the rumino-reticulum. These processes largely determine the release of nutrients for the rumen microbes and the host. They are thus central for the understanding of the performance of ruminant animals (Faichney 1986). Although qualitative aspects of these processes are well understood, emphasis is now being placed on understanding their quantitative contributions. Increasing stress on manipulating rumen dynamics to improve efficiency requires understanding of the quantitative importance of these processes (Ulyatt et al. 1986).

Until now, the best estimates of the rates of clearance of particles from the rumen have been made by using indigestible or undigested material. However use of such material does not yield a reliable estimate of outflow of digestible nutrients, which probably would be intermediate between the flow pattern of fluids and solids (Church 1979). Cr mordant fulfills most of the desirable criteria as a particulate marker. It is a stable marker of solids forming hexacoordinate ligands with hydroxyl groups that are very difficult to hydrolyse (Uden et al. 1980). Cr

mordanted to fibre is the most tenaciously bound of the particulate markers which have been examined (Ellis et al. 1982). Until now, mordanted Cr has been used as marker only at high concentrations. Concentrations of Cr in excess of 80 mg g^{-1} dry matter (DM) render plant cell walls essentially indigestible (Colucci 1979) and as the content of Cr on the fibre decreases, the digestibility of fibre increases. Moreover, Cr bound to feedstuffs at high levels has been found to affect the rate of passage, probably due to altered specific gravity (Ehle 1981).

Respiratory movements are closely associated with reticulum movements during rumination. An extra reticular contraction associated with the regurgitation phase of rumination, is accompanied by an inspiratory effort of greater volume than usual (Church 1979). It would be rational to expect that breathing activity is also coordinated with other more general movement phases of the forestomachs and that breathing would thus influence the onward flow of digesta from the rumino-reticulum to the postruminal tract. However, this possibility does not appear to have been examined experimentally.

Means of investigating the propulsion and movement of digesta within the ruminant stomach have not been readily forthcoming. Observations can be made directly through open fistulas but the information obtained in this manner is limited. Observations have been made using radiographic

techniques but the degree of resolution is quite limited. New approaches include high resolution X-rays (Wyburn 1980) and more recently in our laboratory the use of fiber-optics endoscopy (McBride et al. 1983). However, until now, fiber-optics endoscopy has not been used to investigate the general movement of digesta within the rumino-reticulum.

The physical nature of the feed appears to be a most important factor controlling exit from the rumen. Specific gravity and buoyancy are two important physical properties of feed. Schalk and Amadon (1928) felt that specific gravity of solid ingesta was a determining factor as regards the route to be followed from the cardia to the reticulo-omasal orifice. The effect of specific gravity on the mean retention time of particles in the rumino-reticulum may result from the rate at which the particles separate from the mass of digesta, sink into the fluid layer in the ventral rumen and reticulum and so pass to the omasum. Thus, there is faster passage out of the rumino-reticulum as the specific gravity increases from 1.02 to 1.21. Particles of specific gravity 1.40 are retained in the rumino-reticulum for a longer time than particles of specific gravity 1.21 (Campling and Feer 1962).

The nutritive value of feeds and forages is greatly affected by physical form and other physical attributes (Van Soest 1982). It is probable that the shape and nature of the particles are related to their chemical composition.

Ehle and Stern (1984) stated that the distinction between chemical and physical characteristics of feedstuffs is usually vague, since chemical composition often ultimately determines the physical attributes of feed (e.g., density and particle shape).

The present practice of describing forage cell wall constituents as a broad category including cellulose, hemicellulose and lignin is crude and may mask significant underlying physical and chemical differences. Further research in this field is needed. Progress may first require utilization of more refined analytical techniques (Ehle and Stern 1984). Nuclear magnetic resonance (N.M.R.) is an example of such a technique (Elofson et al. 1984).

There is a limited understanding of factors affecting forestomach motility (Waghorn 1977). An understanding of these factors is important since any marked changes in motility might be expected to affect the efficiency of digestion in the forestomach. The summed excitatory and inhibitory effects of afferent inputs to the gastric motor centers play a major role in determining the pattern of motility exhibited by the reticulum and rumen at any given time (Comline and Titchen 1961). Gastric motor centers are affected not only by afferent inputs from the gut but also by the activity of higher centers in the brain which may be influenced by psychological stress. Sensory inputs arising from environmental stimuli, such as temperature, may also influence the gastric centers. Cold exposure has been

observed to increase frequency of reticulum contraction in sheep (Westra and Christopherson 1976) and in cattle (Gonyou et al. 1979). However, these studies were done after a relatively long adaptation to the cold environment. Little information is available regarding the effects of acute cold stress on gut motility of steers. Moreover, little is known about the interaction of stresses with different types of diets.

The first objective of the studies described herein was to create a new quantitative particle kinetic model by using particles mordanted with Cr at very low concentrations. The second objective was to investigate timing relationships between respiration movements and the normal contraction sequence of the ruminant forestomachs. The third objective was to determine the usefulness of a fiber-optics endoscope in visualizing the kinetic characteristics of particulate rumino-reticulum phase. The fourth objective was to investigate the interrelationship between buoyancy, shape, size and chemical composition of particulate matter. Finally, the last objective was to determine the effects of different types of diets and stresses on reticulum, rumen, and omasum contraction frequencies.

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II. A NEW APPROACH FOR A QUANTITATIVE SCHEMATIC KINETIC MODEL OF PARTICLE DEGRADATION AND PASSAGE IN THE RUMINANT DIGESTIVE TRACT USING LOW CONCENTRATIONS OF ^{51}Cr.

A. INTRODUCTION

Clearance of feed residues from the rumino-reticulum has long been recognized as a major process determining and, therefore, controlling both the intake and digestion of forages. Clearance depends on two interacting processes: the first being breakdown through the physical reduction in particle size plus microbial digestion, and the second being physical passage from the rumino-reticulum. These processes largely determine not only the release of nutrients for the rumen microbes and the host, but also the amount of forage that can be eaten. They thus play a central role in determining the productive performance of ruminant animals (Faichney 1986). Although the qualitative aspects of these processes are well understood, more emphasis needs to be placed on understanding quantitative aspects. The increased use of mathematical models to examine control processes and the increasing interest in manipulating rumen dynamics to improve efficiency depends on understanding the quantitative importance of these processes (Ulyatt et al. 1986).

Until now the best estimates of the rates of clearance

of particles from the rumen have been made by using indigestible or undigested material. However use of such material does not yield a reliable estimate of the outflow of digestible nutrients, which probably would be intermediate between the flow pattern of fluids and solids (Church 1979).

Chromium mordant fulfills most of the desirable criteria as a particulate marker. It is a stable marker of solids forming hexacoordinate ligands with hydroxyl groups that are very difficult to hydrolyse (Uden et al. 1980). Chromium mordanted to fibre is the most tenaciously bound of the particulate markers which have been examined (Ellis et al. 1982).

Concentrations of Cr in excess of 80 mg g^{-1} dry matter (DM) render plant cell walls essentially indigestible (Colucci 1979); as the content of Cr on the fibre decreases, the digestibility of fibre increases. Moreover, Cr bound to feedstuffs at high levels has been found to affect the rate of passage, probably due to altered specific gravity (Ehle 1981).

The objective of this work was to evaluate low-level Cr mordanting of neutral detergent-extracted forage particles as a means to attain biologically realistic measurements of particle kinetics in the rumen.

B. MATERIALS AND METHODS

Animals, Diets and Management

Four 15 month-old Hereford steers, weighing 400 - 409 kg, were prepared with rumen fistulas of 10 cm diameter. Each animal was maintained in an individual metabolism crate with continuous lighting at ambient temperatures of 20 - 22^{0}C during the experiment and for 2 weeks before the experiment. Bromegrass hay (Bromus inermis) chopped through a 76 mm screen and harvested at mid-to-late bloom was offered at 2 hour intervals using an automatic feeding device. Body weight was maintained by providing 450 g of hay DM each 2 hours. The chemical composition, per kg DM, of the diet was 13.9 g N, 672 g cell wall constitutents (neutral detergent fibre, NDF), 349 g acid detergent fibre (ADF) and >900 g large particles (those retained on screens of aperture 3.35 mm and larger after wet-sieving, (Dixon and Milligan 1985). Cobalt mineralized salt blocks (Canadian Salt Ltd., Montreal, Canada) and water were available ad libitum.

Preparation of the Cr Mordanted Particles

The leafy material was first separated from the stems. Half of the stems were ground in a Wiley Mill grinder with a screen aperture of 2 mm (small particles, SP), and the

other half were cut at 1 cm lengths (long particles, LP). The ground stems were then sieved by a wet-sieving procedure (Dixon and Milligan 1985) that allowed the particles to pass through a 2 mm screen but not through a 1 mm screen. The particles were immersed in acetone for 24 hours, in methanol for 24 hours to dissolve the cuticle, and then washed in boiling neutral detergent. The stems were finally rinsed with distilled water.

The neutral detergent extracted brome grass stems were mordanted with ^{51}Cr-containing Cr using a modified mordanting technique (Uden et al. 1980). The amounts of Cr used in mordanting were equivalent to 0.5% and 0.01% of the fibre weight and the heating time was 3 hours. The dry, mordanted stems were finally washed in boiling neutral detergent for 1 hour and then washed thoroughly with tap water until the solution was colourless. The quantities of ^{51}Cr used were 1 mCi/animal in the binding solution for the large particles and 0.75 mCi/animal in the binding solution for the small particles. All the particles were dried at 65 ^{0}C for 24 hours, weighed, and the radioactivity/g DM was determined for each fraction using a gamma spectrometer (Model 8000, Beckman Instruments, Fullerton, California).

Experimental Design and Schedule

The experimental design was a 4 X 4 Latin square. The four treatments entailed long particles (10 mm) or small particles (1 - 2 mm) mordanted with either 0.5% or 0.01%

Cr. The fibres were introduced into the rumen in the following quantities: 24 g for the small particles (SP) and 92 g for the long particles (LP). Equal quantities were placed in six different rumen locations: top and bottom of the reticulum, rumen ventral sac, rumen dorsal sac, top and bottom of the caudal sac. The rumino-reticulum contents were then mixed. Subsequently, grab samples from the same six locations were obtained and thoroughly mixed. The radioactive material was introduced into the rumino-reticulum at the beginning of each week for 4 weeks and the sampling started 2 h after the introduction. One week of rest separated the first two sampling weeks from the two last sampling weeks to allow the radioactivity to decrease and to give the animals a rest. The sampling was done every 2 hours for the first 4 hours, every 3 hours for the following 57 hours, and every 6 hours for the last 72 hours. The feces were sampled from the rectum at the same times.

Stability of Cr Binding and Digestibility of Cr Bound Particles

The efficiency of ^{51}Cr binding was determined by measuring the proportion (%) of radioactivity associated with the stems after being in the radioactive heating solution for various times. The percentage of radioactivity lost in boiling neutral detergent solution after different

times of heating was measured to indicate the stability of ^{51}Cr binding. The digestibility of the mordanted particles was measured by the nylon bag technique (78 h rumen incubation).

Particle size determination

The rumen samples from the steers receiving the large mordanted particles were sieved by the screen-by-screen wet sieving method as described by Dixon and Milligan (1985) with the exception that the up-down cycle was repeated 10 times before repeating the process for the next smaller screen. Screens of 7.74, 4.0 and 3.35 mm apertures were used to separate large particles (>3.35 mm) from small particles (<3.35 mm). The rumen samples from the animals receiving the small mordanted particles were not sieved nor were the faecal samples. All samples were dried at 65 ^{0}C for at least 24 hours and then transferred to plastic scintillation vials. The ^{51}Cr radioactivity/g DM was determined in triplicate using a gamma spectrometer (Model 8000, Beckman Instruments, Fullerton, California).

Eating boluses were collected from the four steers by way of the rumen cannulae to verify the bolus long particle (LP) and small particle (SP) flows to the large particle pool (LPP) and small particle pool (SPP) respectively. The boluses were sieved using the technique described previously.

Calculations and Statistical Analysis

Radioactivity counts (CPM) were converted to a per g DM basis and then transformed to natural logarithmic values. These values were plotted against time after dosing with ^{51}Cr and linear regression equations were calculated by using SPSSX programs. Univariable equations of type Y = Intercept - slope X were used to calculate the values for turnover time (TT), pool size (A) and flow (a); as described by Shipley and Clark (1972) for a first order kinetic pool. The analysis of variance for TT, A, and a were done using SPSSX program. Subsequently, a quantitative rumino-reticulum model was developed for forage particles. The model values were determined as follows:

1) Feed intake : weight of DM given = 100%
2) Flow from the eating bolus to LP Pool (LPP) : same as flow (a) for the LP (verified by sieving the bolus)
3) Flow from the eating bolus to SP Pool (SPP) : (Flow out of SPP) - (Flow toward SPP from LPP); (verified by sieving the bolus)
4) Soluble component : (Feed) - (flow to LPP + Flow to SPP)
5) LPP size : A for LP (verified by sieving)
6) SPP size : A^1 for SP (verified by sieving)
7) Flow out of LPP : "a" of LP
8) Flow out of SPP : "a^1" of SP

9) Flow toward SPP from LPP : obtained by comparing the area under the curve of the SP originating from LP to the surface under the curve of LP disappearance.

10) Volatile fatty acids (VFA) + CO_2 and CH_4 from LPP : Flow out of LPP - (Flow toward SPP from LPP + flow of large particles directly out of the rumino-reticulum (RR)).

11) Flow of LP which go directly out of the RR : (%) of the total feces DM weight formed by LP DM weight (obtained by wet sieving the feces).

12) Flow out of the RR from SPP : Obtained by Grovum & Williams (1973) procedure in animals dosed with SP.

13) VFA + CO_2 and CH_4 from SPP : flow out of SPP - Flow out of RR from SPP

14) Small intestine flow : (soluble component escaping rumen fermentation ?) + (Flow of LP which goes directly out of the RR) + (Flow out of RR from SPP)

15) Digestibility of DM in the (RR) : DM - flow into small intestine (?)

16) Post-ruminal digestibility : DM - (digestibility in rumen (?) and residual in feces)

17) Total DM digestibility : DM feed - DM feces.

18) Post-ruminal turnover time : obtained by the procedure of Grovum and Williams (1973).

Analytical technique

Cell wall contents (CWC) were determined by the procedure of Goering and Van Soest (1970), and N was determined by the Kjeldahl method (AOAC 1975).

C. RESULTS AND DISCUSSION

Particle Sieving Technique

Several authors have proposed that the particulate DM present in the rumen can be kinetically represented by two pools: a large and a small particle pool (Hungate 1966; Poppi et al. 1981). Although Dixon and Milligan (1985) demonstrated that there was no clearly discernable size below which all particles were equally eligible to leave the rumen a two-pool model involving a small- and large-particle pools (SPP and LPP) is likely to be a useful simplification in describing the kinetics of particulate matter in the rumen. Dixon and Milligan (1985) concluded that for cattle, using the current sieving techniques, the DM retained by the 3.2 mm screen provided an appropriate minimum definition of the large particulate pool because fewer than 15% of the fecal particles will be of this size or larger.

The method of wet sieving used in the present study was described by Dixon and Milligan (1985). The mechanical sieving techniques used in other studies where the orientation of particles and screens may be different (Jones and Mosely 1977; Poppi et al. 1980) could give different results. Consequently, differences in particle size distribution measured using different sieving

techniques may, to a large extent, reflect differences in the basis of selectivity of the techniques rather than actual particle size differences between experiments. For this reason comparisons of particle size distribution with other studies using different sieving techniques should be made with caution (Dixon and Milligan 1985).

Stability Test and Digestibility

The efficiency of binding Cr was inversely proportional to the concentration of Cr in the bathing solution (Table II.1). This indicates that a limited number of sites are available for binding. The percent of associated Cr subsequently lost from the stems in boiling neutral detergent solution is also inversely proportional to the Cr concentration of the mordanting solution used (Table II.1). With increased time of heating during mordanting there was increased extent and tenacity of binding (Table II.1). This longer heating time might favour Cr binding to hydroxyl groups of components more refractive to degradation.

The digestibility of the long and small particles of NDF-extracted brome stems was practically unaffected by mordanting with 0.01% Cr (Table II.2). The fibres mordanted with 0.5% Cr had their digestibility substantially reduced. This is in agreement with Uden et al. (1980) who stated that when the concentration of Cr on mordanted particles

was increased (from 0 to 80 mg g^{-1}DM) the *in vitro* cell wall digestibility decreased drastically.

The loss of ^{51}Cr during rumen incubation was nearly identical to loss of DM (Table II.1). Thus, the label appears to be associated with components of the extracted particles that are removed during digestion. Nevertheless, a question remains unsolved: "Where does the Cr go when liberated by digestion?". One might expect that the Cr would remain bound to very small particles because Cr mordanting forms strong hexacoordinate ligands with hydroxyl groups (Uden et al. 1978). Ellis et al. (1982) stated that feedstuffs mordanted with Cr constitute labelled material in which there is little doubt as to marker location because migration within and between particles is low.

Kinetic regression equations based on rumen sampling

The high R^2 values indicate that LP and SP groups (Table II.4) in the rumen were reasonably represented as homogenous first order kinetic pools. This is in agreement with experiments (Dixon and Milligan 1985) in which disappearance from the rumen of fluid, particulate, and microbial markers, occurs according to first order kinetics. The better fit of small than large particles probably reflects the fact that more particles were introduced into the rumen in the small form and, therefore, sampling would entail less statistical variation. Also, the

mixing of the small particles is likely to be quicker than for the large particles.

Analysis of the Pool Kinetics

Table II.4 indicates that the slopes and the turnover-times were not significantly different between SP and LP whether mordanted with 0.5 or 0.01% Cr ($p = 0.145$, and $p = 0.164$ respectively), and that animal differences explained the majority of the variation between these treatments ($p = 0.038$ and $p = 0.051$ respectively). The LPP size (A) was significantly greater than the SPP size (A^1, $p < 0.001$). The flows out of each pool (a and a^1) were different ($p < 0.01$); with the LPP flow being greater ($p < 0.05$) than the SPP flow.

Particle sizes and Cr concentrations were used in the treatments, consequently, orthogonal contrasts (Table II.3) were necessary to separate the effects of these two factors on the pool kinetic parameters. The TT differences depended on Cr concentrations ($p = 0.067$), while pool sizes and flows were related to the particle sizes ($p < 0.001$). The shorter TT of the particles treated with 0.01% Cr than those treated with 0.5% Cr likely reflects the greater digestibility of the former preparation. The size of, and flow of DM, through the kinetic pool of large particles were roughly twice as great as for small particles therefore the values for TT of SPP and LPP were nearly

equal. These observations are consistent with results of Dixon and Milligan (1985) who found that the LPP was about 60±7% and the SPP was 40±5% of the total rumen particle pool for steers fed long hay.

The fractional turnover rate of the ^{51}Cr-labelled material in the LPP itself would be equal to the sum of the rate constants of the digestion of large particle DM and the mechanical breakdown of the large to small particles, plus the small proportion of large particles which go directly out of the rumino-reticulum. These combined processes sum to a daily turnover rate of 0.88±0.09 for particles mordanted with 0.5% Cr and 0.96±0.1 for particles mordanted with 0.01% Cr for chopped hay. These values are consistent with the observations of Dixon and Milligan (1985) who measured a fractional turnover rate of the rumen large-particle pool of 1.07 d^{-1} for steers given long hay and 0.82 d^{-1} for those given ground hay.

The daily turnover rate of the SPP was 0.85±0.16 d^{-1} for the 0.5% Cr particles and 0.95±0.1 d^{-1} for the 0.01% Cr particles. These results agree with Dixon and Milligan (1985) who observed that the fractional outflow rate (FOR) of lignin in the rumen small particle pool was 1.07 per day. This was similar to the mean FOR (1.17 per day) of the 2.0-3.2, 1.0-2.0 and 0.25-0.5 mm mesh particle groups, and the mean (1.2 per day) of these groups when weighted for their relative pool size in the rumen (Dixon and Milligan 1985).

Rate Constants Derived from the Changes in Concentrations of Marker in the Faeces

Turnover times for label in the rumino-reticulum (TT_1), and the post-ruminal tract (TT_2), transit time for 50% excretion of the marker (D_{50}) and total mean retention time (TMRT) were all significantly affected ($P<0.05$) by particle size, but not by level of Cr treatment (Table II.5).

TT_1 of the small particles (23.6±4.4 h, 0.5% Cr; 22.5±2.5 h, 0.01% Cr) were similar to the estimates obtained by direct sampling from the rumen (28.2±2.8 h, 0.5% Cr; 25.3±2.5 h, 0.01% Cr). However, TT_1 of the large particles as determined from fecal sampling (38.3±12.6 h, 0.5% Cr and, 31.0±5.1 h, 0.01% Cr) were significantly greater than the TT of the large particles calculated from rumen sampling (27.1±2.98 h, 0.5% Cr and, 24.9±2.5 h, 0.01% Cr). The rumen sampling technique, however, yields the turnover time of the overall LP flow including both the breakdown and digestion of the LP and passage of the resulting small particles. The fact that TT_1 was not the exact summation of the TT of the respective large and small particle pool may have been a consequence of the flow of LP which go directly out of the rumino-reticulum. This direct flow of LP out of the rumen results in a lower TT as opposed to the simple addition of

large particle TT and small particle TT.

A major rate-limiting step when clearing dietary residues from the rumino-reticulum is passage through the reticulo-omasal orifice (Ulyatt et al. 1986). Since particle size reduction is a prerequisite for passage through this orifice (Evans et al. 1973; Ulyatt et al. 1976; Welch and Smith 1978; Poppi et al. 1981), it is important to realize that the undigested component of the LPP has a turnover time (TT) for the rumen determined by breakdown of the LPP and passage through the SPP. This has the effect of adding the TT of SPP to the TT of the LPP to give the total rumen TT of the undigested material from large particles.

Analysis of the descending phase of the fecal curve of the SP, originating from the breakdown of the LP, gave an average TT of 47±8 h. Addition of the rumen TT of LP plus the rumen TT of SP yielded an average of 51±5 h (Table II.6). Further emphasis of this similarity is evident in the correlation coefficient of 0.82. This differed from the TT of large particles as calculated from fecal sampling for label added to the rumen as large particles (38.28 h and 31.04 h for LP 0.5% Cr and LP 0.01% Cr respectively) . The difference might be the consequence of a direct exit of large particles through the reticulo-omasal orifice. Thus, the calculation of the disappearance of the small particles originating from the large particle pool resulted in a value similar to addition of the rumen TT of the large

particle pool plus small particle pool. This is an indication of the accuracy of the technique.

The size of the rumen particles had a significant effect ($p < 0.01$) on the TT_2 (Table II.5). The large particles pass more slowly through the hind gut. This was probably due to the large particles which passed directly out of the rumen.

The concentration of Cr, and consequently a change in the digestibility of the particles, did not appear to have any significant effect on the turnover times, D_{50}, or TMRT (Table II.5). On the other hand, the latter three values were larger ($p<0.01$) for the large particles than for the small particles. This is in agreement with Van Soest (1982) who stated that particle size per se tends to have its own effect on passage, smaller particles passing faster than larger ones.

Quantitative Model (0.01% Cr)

A kinetic model assembled from data derived from the particles subjected to 0.01% Cr mordanting is presented as Fig. II.1. Some 20% of the DM of the eating bolus was soluble. DM flow to the LPP represented 63±15% of total DM or 85±19% of the particulate DM of the eating bolus. The DM flow directly to SPP represented 12±5% of the total DM or 15±6% of the bolus particulate DM. The conclusion that DM of the eating bolus was composed of 80% particulate matter

and 20% soluble component is in agreement with Ulyatt et al. (1986) who stated that approximately 35% of the DM of fresh forage is solubilized by chewing, while only 20-30% is released from dried diets. There were large quantitative differences in the results of experiments measuring the particle sizes of the eating bolus in the literature; these can be explained in terms of the sieving techniques used (Ulyatt et al. 1986). Nevertheless, chewing during eating appears to be very efficient in releasing the soluble components. The first order kinetic calculations indicated that the LPP represents 70±13% (3815 ± 470 g) and the SPP 30±11% (1632 ± 180g) of the particulate DM in the rumen. When samples of rumen contents were wet-sieved, 65±5% and 33±3% of particulate DM were measured to be in large and small particles respectively. Thus, the calculated values were in agreement with the sieving technique results.

The flow of DM out of the LPP is divided between the production of VFA, CO_2 and CH_4 (46±22% of the original feed DM), LP which go directly out of the reticulo-rumen (4.6±1.7%), and LP which go to SPP (17.0±7.1%). Fermentation products accounted for 68% of the DM flow out of the LPP. Flow from the LPP constitutes 59% of flow into the SPP. Flow of large particles directly out of the rumino-reticulum is relatively small (6.8±2.5% of the DM flow out of the LPP), which is in agreement with Dixon and Milligan (1985). These authors noted that only

10.7 - 15.3% of the faecal particulate DM was retained by 3.2 mm mesh and larger screens. This is in agreement with our observation that 15.5±5.7% of the particulate DM in the feces is large particles (>3.2 mm).

The present data does not yield an estimate of VFA, CO_2 and CH_4 produced by the fermentation of the soluble components. However, one might expect that a part of the soluble components originating from the eating bolus may pass readily out of the rumino-reticulum and be absorbed in the lower digestive tract. Van Soest (1982) stated that fine, soluble and liquid matter escape the rumen more rapidly than coarse, light, solid matter. Dixon and Milligan (1985) noted that the FOR of even the smaller rumen particle groups is considerably less than that of water. Time of fermentation may limit the extent of digestion in the rumen (Owen and Goetsch 1986). Some of the soluble fraction would be assimilated by rumen microbes and pass through the reticulo-omasal orifice in this form.

The DM flow from the SPP was comprised of VFA, CO_2 and CH_4 (4±5% of the original feed DM or 14±17% of the total DM of this pool) and flow out of the rumen (25±7% of the original feed or 86±24% of the total DM of this pool). The VFA plus CO_2 produced in this way are relatively small, but this is logical because about 60% of the SPP originates from the LPP, and consequently has already been subjected to fermentation. On the other hand, it would be expected that the intensity of microbial attack on the

small particles would be greater due to a larger surface per unit weight. Nevertheless, Pond et al. (1984) stated that simple reduction in particle size does not mean the material will be more digestible, or that the rate of digestion will be greater. Akin and Burdick (1981) found that many small particles were deeply stained with acid phloroglucinol, indicating a high content of lignin and low digestibility in which case no increase in digestibility would be predicted from further particle size reduction or increased surface area exposure.

According to the kinetic calculation of this study, material leaving the rumino-reticulum consisted of large particles, small particles and soluble components that were equivalent to 5, 25 and 0-20% of original feed DM. These results are consistent with the idea that the relative resistance of DM to passage is related to size (Poppi et al. 1980; Weston and Cantle 1984). Clearance rate within diets approaches zero for the largest particle fraction and progressively increases as the fractions become smaller (Weston and Kennedy 1984).

The digestibility results indicate that the rumen is the principle site of dietary DM digestion. The small intestine and the lower digestive tract absorb less than half of the amount of particulate DM digested in the rumino-reticulum. Only 31.7±3.8% of the feed DM was found in the feces. This is consistent with the results of Dixon and Milligan (1985) who assumed in their calculations that

0.80 of the total DM digestion occurred in the rumen. The proportion of the total digestible organic matter digested in the rumen in cattle given forage diets has been estimated as 0.71 - 0.82 for oaten chaff (Redman et al. 1980), 0.79 for wheaten straw (Srisbandarajah et al. 1982), 0.82 - 0.89 for spear grass hay (Hunter and Sieberg 1980) and 0.80 - 0.93 for mixed grass legume hay (Kennedy 1982).

The apparent DM digestibility of the hay was 68.3±3.8%. This is in agreement with Kennedy (1985) who found an apparent digestibility of 72±2.3% for chopped bromegrass hay in sheep exposed to ambient temperatures of 22 - 25^{0}C.

Comparison Between the 0.01% Cr and the 0.5% Cr Models

Comparison of kinetic models (Fig. II.1 and Fig. II.2) for the two concentrations reveals that there was a greater proportion of soluble components, less LP, and the same portion of SP in the 0.5% Cr model as compared to the 0.01% Cr model. On the other hand, the SPP was larger. Less VFA plus CO_2 and CH_4 were produced from the LPP. The large particles which go directly out of the rumino-reticulum and the flow toward the SPP from the LPP were both increased in the 0.5% Cr model. Flow out of the SPP and flow out of the rumen from the SPP are similar between the two concentrations as well as the VFA plus CO_2 and CH_4 production from the SPP. The VFA and CO_2 and CH_4

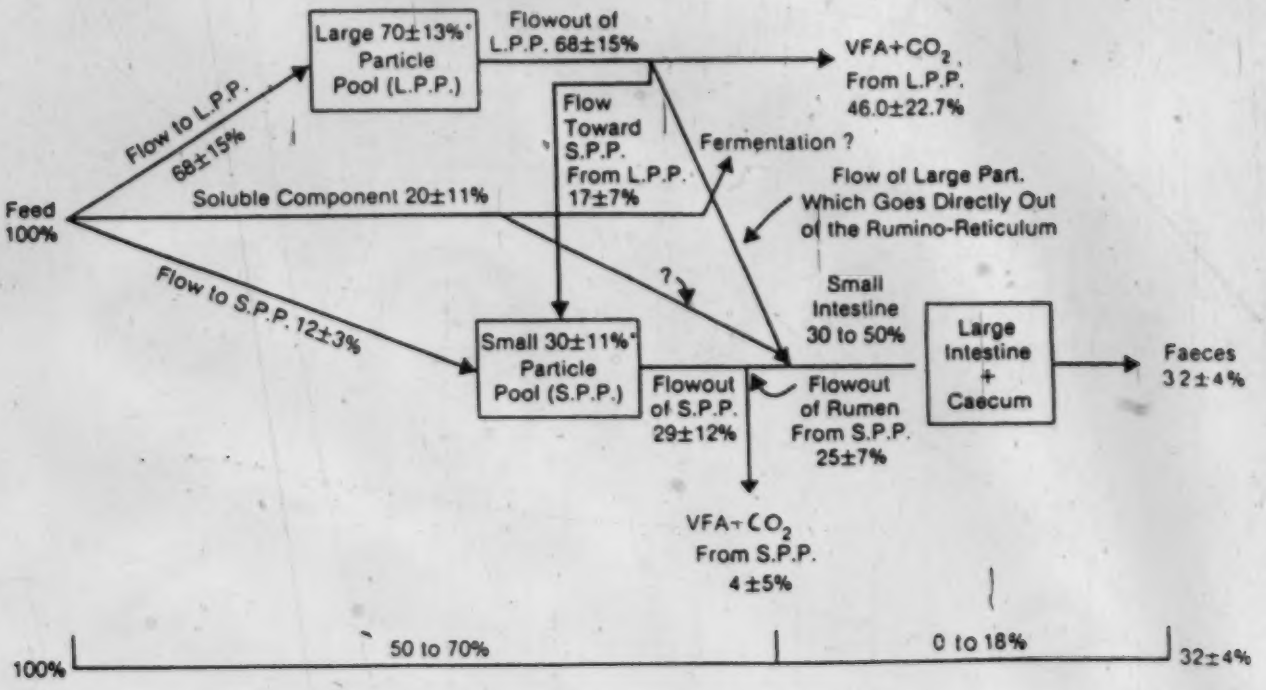
produced from LPP were less for the 0.5% Cr model. The main differences between the two models appeared to be as a consequence of a lower digestibility of the 0.5% Cr particles. The differences show how marker concentrations can affect values obtained. The other kinetic values seem to be relatively similar considering the high standard deviations.

The large standard deviations indicate differences between animals in particle size reduction by chewing during eating. This has been found by Ulyatt et al. (1986) who stated that there are consistent differences between animal in the effectiveness of chewing.

The fermentation products in the rumino-reticulum (VFA, CO_2 plus CH_4) represented 72% (0.01% Cr Model) and 61% (0.5% Cr Model) of the total feed digestibility. This is supported by Sutton (1972) who stated that production of volatile fatty acids (VFA) in the rumen fermentation provides between 50 and 70% of the total digestible energy.

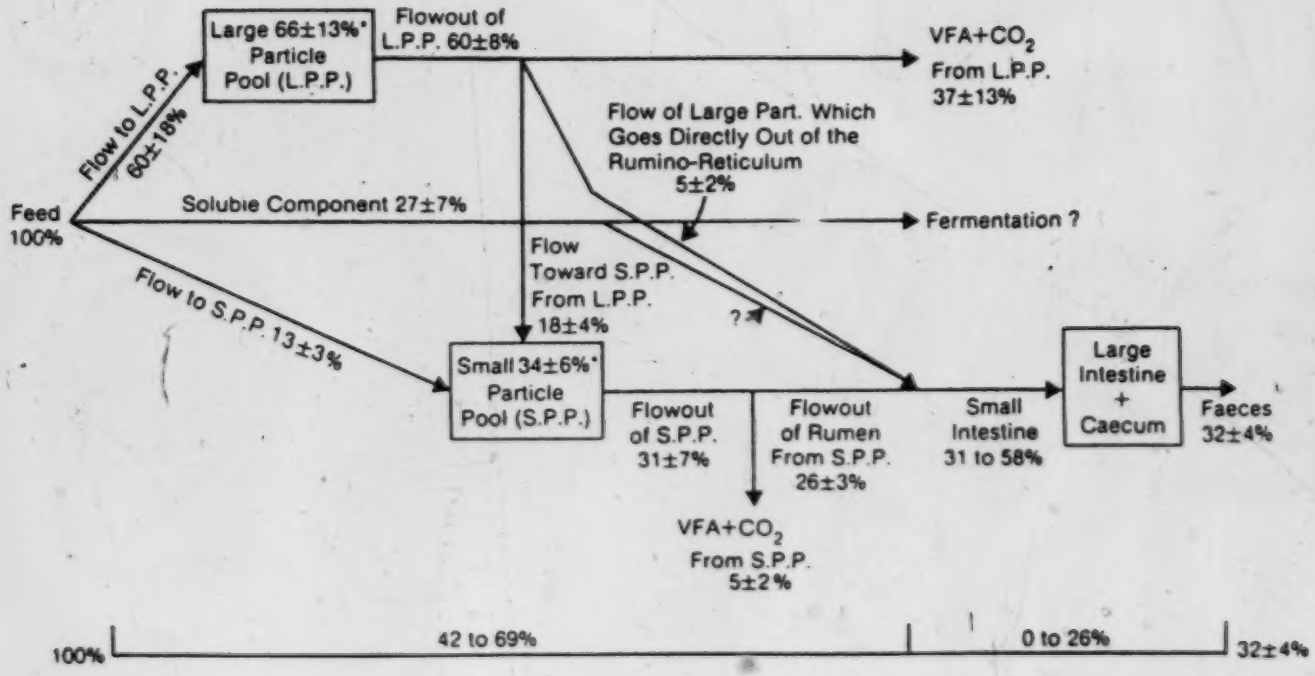
This model is a simplification of the multiple digestive processes acting in the ruminant animal. The quantitative technique seems to measure, with relative accuracy, the different digestive functions. Nevertheless, more work remains to be done to improve the actual descriptive field which was limited to NDF extracted bromegrass stems.

Fig. II.1. Quantitative kinetic model of ruminant digestive tract with ^{51}Cr mordanted feed particles at a low concentration (0.01% Cr).



* Percent of the Total Particulate Matter in the Rumino-Reticulum.

Fig. II.2. Quantitative kinetic model of ruminant digestive tract with ^{51}Cr mordanted feed particles at a low concentration (0.5% Cr).



* Percent of the Total Particulate Matter in the Rumino-Reticulum.

Table II.1: Binding of different concentrations of Cr mordant on NDF extracted brome stems.

a) BINDING	Cr in Solution (% of DM)	Time of Heating (h) 1	3	24
Efficiency of binding (%)[1]	10	17.8	35.5	70.4
	2.0	27.8	47.1	72.6
	1.5	27.0	48.3	82.8
	1.0	29.9	53.9	88.9
	0.5	36.8	68.4	90.1
	0.01	51.2	84.2	91.0
% of bound Cr^{51} lost in boiling neutral detergent solution	10	14.1	8.4	6.8
	2.0	26.8	14.2	12.5
	1.5	24.8	14.1	11.5
	1.0	26.8	14.6	10.6
	0.5	38.5	24.4	16.9
	0.01	37.1	28.6	29.9

[1] Efficiency of binding was determined by measuring the radioactive proportion (%) of the radioactive heating solution associated with the stems after leaving the stems in this solution for various times.

Table II.2: In situ digestibility (72 h) of non-mordanted and mordanted particles at different Cr concentrations.

Size of particles	Treatment Cr (%)	Digestibility (%) ± S.D.	Loss of ^{51}Cr (%) ± S.D.
Long	0	31.28 ± 1.80	
Long	0.01	29.05 ± 2.30	34.3 ± 5.7
Long	0.5	20.24 ± 8.53	22.0 ± 7.1
Small	0	49.85 ± 2.92	
Small	0.01	47.73 ± 8.16	50.5 ± 6.0
Small	0.5	31.97 ± 6.43	33.2 ± 8.0

Long particles: Particles of 10 mm long.
Small particles: 1 mm < particles < 2mm
S.D.: Standard Deviation.

Table II.3: Kinetic regression equations based on rumen sampling.

Stem sizes and Cr Concentrations	Equations	R^2	R^2 S.D.
Long particles 0.5% Cr	8.87933 - 0.0369 X = Y	0.91276	0.04753
Small particles 0.5% Cr	8.64632 -0.0363 X = Y	0.981535	0.00997
Long particles 0.01% Cr	8.97530 - 0.0405 X = Y	0.91824	0.01949
Small particles 0.01% Cr	8.91737 - 0.0398 X = Y	0.985478	0.00421

S.D.: Standard deviation.
X : Independent variable (Time).
Y : Dependent variable (Radioactive Count per minute).

Table II.4: Analysis of variance and orthogonal contrasts on pool kinetic values based on rumen sampling.

	Treatment1 Long part. 0.5%	Treatment2 Small part. 0.5%	Treatment3 Long part. 0.01%	Treatment4 Small part. 0.01%	Main effects Sig. F	Source of variance Significance of F Treat.	Week	Animal
Slope ($X10^{-2}$)	3.69	3.63	4.05	3.98	0.145	0.308	0.753	0.038
TT (h)+	27.13	28.20	24.91	25.27	0.164	0.250	0.802	0.051
A (g)++	3724.00	1929.50	3815.75	1632.00	0.009	0.061	0.132	0.416
a ($g\ h^{-1}$)θ	135.60	69.85	154.61	65.84	0.030	0.004	0.231	0.987
R^2	0.91	0.98	0.89	0.99	-	-	-	-

ORTHOGONAL CONTRASTS[1]

	Slope	TT	Pool Size (A)	Flow (a)
0.5% vs 0.01% Cr	0.083	0.067	0.698	0.566
Small vs. long particles	0.716	0.609	0.000	0.001

+TT: Turnover Time.
++A: Size of the pool.
θa: Flow
[1]Significance of the orthogonal contrast which compared both Cr levels and both sizes of particles.

Table II.5: Analysis of variance and orthogonal contrasts on pool kinetic values based on feces sampling.

	Treatment1 Long part 0.5%	Treatment2 Small part 0.5%	Treatment3 Long part 0.01%	Treatment4 Small part 0.01%	Main effects Sig. F	Source of variance significance of F Treat.	Week	Animal
TT_1 (h)+	38.28	23.61	31.04	22.50	0.047	0.021	0.726	0.045
TT_2 (h)++	22.67	15.96	24.32	14.11	0.002	0.000	0.377	0.093
Transit (h)@	10.68	24.30	10.39	14.69	0.001	0.000	0.019	0.008
D 50 (h)-	50.08	32.89	46.05	30.40	0.004	0.001	0.560	0.056
TMRT (h)#	71.62	58.70	65.51	51.43	0.011	0.002	0.819	0.034
TMRTS	46.66	43.22	45.43	41.75	0.011	0.005	0.083	0.032
R^2	0.88	0.72	0.91	0.86	-	-	-	-

ORTHOGONAL CONTRASTS[1]

	TT_1	TT_2	Transit	D50	TMRT	TMRTS
0.5% vs 0.01% Cr	0.179	0.901	0.883	0.117	0.115	0.067
Small vs. long Particles	0.005	0.000	0.000	0.000	0.000	0.001

+TT1: Turnover Times for label in rumino-reticulum.
++TT2: Turnover Time for label in post-ruminal tract.
-D_{50}: Transit time for 50% excretion of the marker.
#TMRT: Total mean retention time.
[1]Significance of orthogonal contrasts which compared both Cr levels and both sizes of particles.

Table II.6 Turnover time (TT) obtained by analysis of the descending phase of the fecal curve of the small particles, originating from the breakdown of the long particles and, TT of large particles and small particles from the rumen sampling and their summation.

Cr Concentration in solution	Animals no.	TT of LPP from feces (h)	TT of LPP from rumen (h)	TT of SPP from rumen (h)	Summation from rumen (h)
0.01%	1	56	25	29	54
0.01%	2	51	24	28	42
0.01%	3	54	26	23	49
0.01%	4	35	21	23	44
0.5%	1	48	25	21	52
0.5%	2	50	29	31	60
0.5%	3	48	26	23	49
0.5%	4	36	24	21	45
Mean:		47±8	25±2	25±4	51±5

TT: Turnover Time.
LPP: Long particle pool.
SPP: Small particle pool.

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III. RELATIONSHIP BETWEEN BREATHING AND MOVEMENTS OF THE RUMINANT STOMACH

A. INTRODUCTION

Respiratory movements are closely associated with reticulum movements during the rumination process. An extra reticular contraction associated with the regurgitation phase of rumination is accompanied by an inspiratory effort of greater volume than usual (Church 1979). This results from a sharp contraction of the diaphragm causing a negative pressure in the trachea, which has been observed to occur in animals with a tracheal cannula (Webster and Cresswell 1957). This would be accompanied by a negative pressure in the thoracic cavity, and an increased anterior force on fluids in the region of the cardia. It would seem rational to expect that breathing activity is also coordinated with other more general movement phases of the rumen-reticulo-omasal complex and would, thereby, influence the onward flow of digesta from the rumino-reticulum to the postruminal tract. However, this possibility does not appear to have been examined experimentally. The objective of this study was to investigate timing relationships between respiration movements and the normal contraction sequence of the ruminant stomach.

B. MATERIALS AND METHODS

Experiment 1

Animals

Four 15-month old Hereford steers (400 to 409 kg body weight) were prepared with rumen cannulas of 10 cm. diameter. Each animal was maintained in an individual pen sufficiently large (3 x 4 m) to permit unrestricted movement. The stable temperature was 21 ± 1°C. Bromegrass hay (Bromus inermis), chopped through a 76 mm screen and harvested at the mid-to late-bloom stage, was offered in twelve equal portions at 2-hour intervals by means of an automatic feeding device. The daily ration of 5.0 kg dry matter (DM), 69.5 g N, per steer was calculated to meet the maintenance requirement (NRC 1984) of the steers. Cobalt-iodized salt blocks and water were available ad libitum.

Experimental Procedures

Each steer was brought into a recording area where they were restrained in a cattle squeeze. Catheters (polyvinylchloride, 0.090 mm i.d.) were placed in the following sites in the lumen of the stomach area via the rumen fistula: rumen (main ventral sac), reticulum, and omasum. The omasum catheter was fitted with an umbrella-shaped, plastic holder of approximately 1 cm diameter in order to anchor the catheter inside the organ. The ends of the rumen and reticulum catheters were fitted with weights to hold them in place. These catheters were

connected to pressure transducers (Gould Statham) to record pressure in the gut lumen. Fluid was maintained in the catheters by means of constant infusion of water (Ismatec pump MT-13 GJ-4) at a rate of 75 mL h^{-1}. Respiration movements were recorded by means of a pneumograph placed around the thorax of the animal and a pressure transducer. The pressure transducers were coupled to a physiological recorder (Beckman model R612). Basal recordings of all parameters were attained between 09:00 and 13:00 hours.

For purposes of analysis, the respiratory cycle was divided into four phases: the inspiratory phase, the maximal zone (between inspiration and expiration), the expiratory phase, and the minimal zone (between expiration and inspiration). Forty to fifty consecutive reticulum contractions were observed for each animal, except for one steer, for which recordings of only twenty consecutive cycles were achieved. The recordings were examined for coincidence between the second peak of the reticulum contraction and each phase of respiration. Possibilities of relationships between breathing and other forestomach movements were also considered. The data were expressed as a per cent of the total number of contractions occurring in each respiratory phase. Analysis of variance using a completely randomized design was employed. Treatment means were compared using Duncan's Multiple Range Test ($p<0.01$).

Experiment 2

Animals

Four 2-year old Suffolk wethers (40 to 46 kg body weight) were prepared with rumen fistulae 3.5 cm in diameter and "T" shaped abomasal cannulae located 3 cm from the pyloric sphincter. Each animal was confined to a metabolic crate (0.9 x 1.8 m). They were housed in a room maintained at a constant temperature (21 $\pm$ 1°C). Pellets of bromegrass (Bromus inermis) hay, harvested at the mid-to late-bloom stage, were offered in twelve equal portions at 2-hour intervals at a maintenance level (1,000 g DM d^{-1}, 13.0 g N d^{-1}) by means of an automatic feeding device (NRC 1975). Cobalt - iodized salt blocks and water were available ad libitum.

Experimental Procedures

Animals were held in their metabolic crates throughout the experiment. Recordings were made of the thoracic respiratory movements and pressure changes in the rumen (ventral sac) and reticulum as described for experiment 1. In addition, recordings of pressure in the abomasum were made by means of a fluid-filled catheter inserted via the abomasal cannula.

The data were analyzed by the statistical procedure described for experiment 1.

Overall Statistical Analysis

A repeated measure statistical design analyzing the

variation between subject factors (the diets and the species differences) and the variation within subject factors (the phases of respiration) was used. The data was standardized (percentage) so sheep and steer totals could be compared (Steel and Torrie 1980).

C. RESULTS

Experiment 1

Fig. III.1 shows a portion of a continuous recording of respiratory and gastrointestinal movements in a steer. For the steers, respiratory frequency was 18.4 $\pm$ 1.5 min^{-1} and the reticulum, rumen, and omasum contraction frequencies were 1.1 $\pm$ 0.1, 1.2 $\pm$ 0.1, and 1.1 $\pm$ 0.1 min^{-1} respectively. Inspiration, maximum, expiration, and minimum phases of respiration accounted for: 39 $\pm$ 10 %, 10 $\pm$ 3 %, 40 $\pm$ 11 %, and 11 $\pm$ 2 % of breathing cycle time. The proportion of second reticular contractions coinciding with each phase of respiration is shown in Table III.1. In these steers, 68.1 $\pm$ 8.7% of the second peaks of biphasic reticulum contractions occurred during the inspiration phase of respiration (Table III.1), only 7.5 $\pm$ 3.1% of peaks coincided with the expiratory phase ($p<0.01$). The percentages of the second peak of biphasic reticulum contraction occurring during the maximum and minimum phases of the respiration cycle differed ($p<0.01$) from those of the inspiratory and expiratory phases (Table III.1) but not ($p<0.05$) from each other. Relative contraction frequencies which relate the occurrence of second contractions during a

respiration phase to the proportion of time spent on that phase, revealed (Table III.2) that contraction frequency was distinctly enhanced during inspiration but it was also slightly enhanced during the maximum phase and it nearly stopped during expiration. The second peak of reticulum contraction corresponded to the beginning of the increase of pressure in the omasum, that is, the beginning of omasum contraction (Fig III.1). Primary contractions of the rumen (main ventral sac) followed second peaks of reticulum contractions by 36 $\pm$ 3 seconds.

Experiment 2

Fig. III.2 shows a portion of the continuous recording of respiratory and gastrointestinal movements in a sheep. Respiratory frequency of the sheep was 10 $\pm$ 3 min^{-1} and the frequencies of reticulum and rumen contractions were 1.7 $\pm$ 0.1 and 2.0 $\pm$ 0.1 min^{-1} respectively. Inspiration, maximum, expiration, and minimum accounted for 38 $\pm$ 12 %, 15 $\pm$ 2 %, 35 $\pm$ 11 %, and 12 $\pm$ 2 % of the time of the respiration cycle. In these sheep, 65 $\pm$ 12% of second peaks of the biphasic reticulum contractions occurred during an inspiration phase of respiration (Table III.1); only ($p<0.01$) 3.4 $\pm$ 3.0 % of the peaks coincided with the expiratory phase of breathing. As was found for steers, there was no difference ($p>0.05$) between percentages of second peaks of biphasic reticulum contractions occurring during either the maximum or minimum phases of the respiration cycle, but in each case incidence differed

($p<0.01$) from those of inspiratory and expiratory phases (Table III.1). Relative contraction frequencies (Table III.2) revealed patterns nearly identical to those for cattle with intensity being substantially greater during the inspiration phase than during the minimum and expiration phases. Intensity was slightly enhanced during the maximum phase. Contractions of the omasum were not recorded in this experiment. There were slow continuous peristaltic movements of the abomasum that did not indicate any obvious relationship with respiration, or the changes of pressure in the other stomach compartments (Fig. III.2).

A summary of patterns of breathing and pressure changes in ruminant stomach locations (Fig. III.3) shows the timing of regular movements of the gut and during respiration. Contractions of the reticulum, particularly the second phase, are reasonably coordinated with an inspiratory phase of breathing and precisely coordinated with opening of the reticulo-omasal orifice. Following this, the omasum immediately starts a biphasic increase in pressure. During these processes, the rumen was relatively relaxed and the abomasum showed no particular relationship with the other compartment movements.

D. DISCUSSION

If occurrence of the second phase of normal reticular contractions was random within the respiration cycle,

distribution would have been approximately 38, 12, 38, and 12 % during each of the inspiration, maximum, expiration, and minimum phases respectively. Results for the inspiration and expiration phases show a clear departure from the expected distribution. Although any comparisons between cattle and sheep were confounded by diet differences in our experiments, coincidence between occurrence of second biphasic reticulum contractions and inspiration was remarkably similar for the two experiments. Conversely lack of these contractions during expiration was also notable.

Relative contraction frequencies indicated a possible neural inhibition of reticular contractions and a restraint of initiation of the forestomach movement cycle during expiration. Inspiration appeared to produce a neural facilitation on the nervous system controlling reticulum contractions. We would view the intermediate respiration phases (maximum and minimum) as transition states between the two phases (inspirations and expirations) that clearly relate to reticulum second contractions.

The reason for a relationship between an inspiratory phase of respiration and second peaks of reticulum contractions is not clear. However, it appears that during second reticular contractions, the reticulum muscle wall and the juxtaposed diaphragm could act in synergy (Fig. III.4) during their contractions to facilitate propulsion

of reticulum contents through the reticulo-omasal orifice, which opens at this time (Bueno and Ruckebusch 1974). This in turn would fill the omasum, which then starts to contract. At that time, a succession of tonic waves in the omasal canal has an aspiratory effect because of its coincidence with the cyclic relaxation of the reticulo-omasal-orifice (Laplace 1970). The tracing of the omasum pressure showed a biphasic pattern. This observation confirms work reported earlier (Stevens et al. 1960) that proposed a two stage functioning of the omasal body; the first being aspiration and filling of the omasal body followed by omasal contraction.

Evidence that transfer of digesta from the reticulum most likely occurs at the time of second peaks of the biphasic contractions is supported by work of Balch et al. (1951). These authors stated that passage of digesta from the reticulo-rumen is largely controlled by a valvelike action of the omasum. Bueno (1972) showed that flow of ingesta into the omasum seemed mainly to be limited by the narrowness of the reticulo-omasal orifice, for it could be doubled by artificially keeping the orifice open. During inspiration, physical pressure of the diaphragm on the abdominal cavity would be expected to exert a force on the reticulum. During contraction of the diaphragm, the reticulo-ruminal fold is contracted, the reticulo-omasal orifice is open (Bueno 1972) and omasal pressure low, therefore fluid will flow from the reticulum into the

omasum. The omasum is buffered from the diaphragm by the static liver, and would be subject to less direct pressure.

A neurological relationship between breathing and the contractions of the reticulum during rumination was described by Breazile (1971). A rumination center was proposed to lie within the medulla oblongata in the vicinity of the nucleus tractus soliterus and the dorsal nucleus of the vagus nerve. This center is located near the respiratory centers and a rhythmic command center, which controls the motor activity of the ruminant forestomach (Breazile 1971). In view of our results, a synaptic link between these centers (respiratory centers and rhythmic command center) appears possible. Such a connection quite clearly does not apply to the abomasum because there was no relationship of the relatively infrequent contractions of this organ to breathing.

In conclusion, in cattle and sheep second peaks of biphasic reticulum contractions coincided with the inspiratory phase of breathing 64 - 68 % of the time. This was 1.7 - fold greater than random probability. Contractions during the expiration phase were only 10 - 20 % of random frequency. Therefore, respiration and movements of the ruminant forestomach are synchronized, to a considerable extent, during the regular pattern of biphasic contractions, as well as during rumination.

Table III.1: Frequency of Occurence of Second Peak of the Reticulum contractions during Each Respiration Phase (mean ± s.d. % of total second peaks).

Experiment	Species	Respiration Phases				Sig.
		Inspiration	Maximum	Expiration	Minimum	
1	Steer	68.1± 8.7a	12.8±5.6b	7.5±3.1c	11.6±4.1b	**
2	Sheep	64.9±11.7a	18.5±5.2b	3.4±3.4c	13.2±7.8b	**
	Mean	66.5±10.5a	15.7±6.1b	5.4±3.8c	12.4±6.3b	**
Between Species Differences						N.S.

a,b,c Values in the same row followed by the same letter are not significantly different ($p<0.01$). (Duncan Multiple Range Test).
** Treatment means are significantly different ($p<0.01$).
N.S. Treatment means are not significantly different.

Table III.2: Relative contraction frequency[θ]

Experiment	Species	Respiration Phases				Sig.
		Inspiration	Maximum	Expiration	Minimum	
1	Steer	1.7	1.3	0.2	1.1	**
2	Sheep	1.7	1.2	0.1	1.1	**

[θ]Relative contraction frequency = $\frac{\text{\% of 2}^{nd}\text{ contractions in phase}}{\text{\% of time spent in phase}}$

** Treatment means are significantly different ($p<0.01$).

Fig. III.1: Relationship between breathing and forestomach pressures in steers

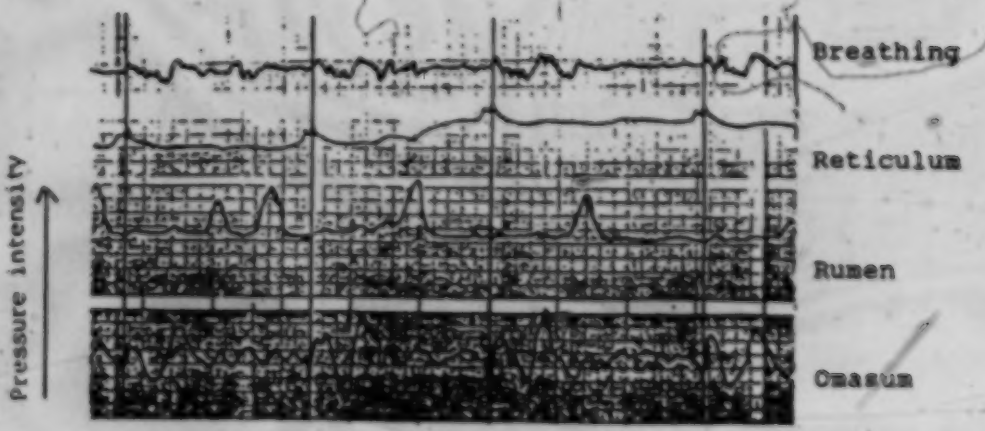


Fig. III.2: Relationship between breathing and forestomach pressures in sheep

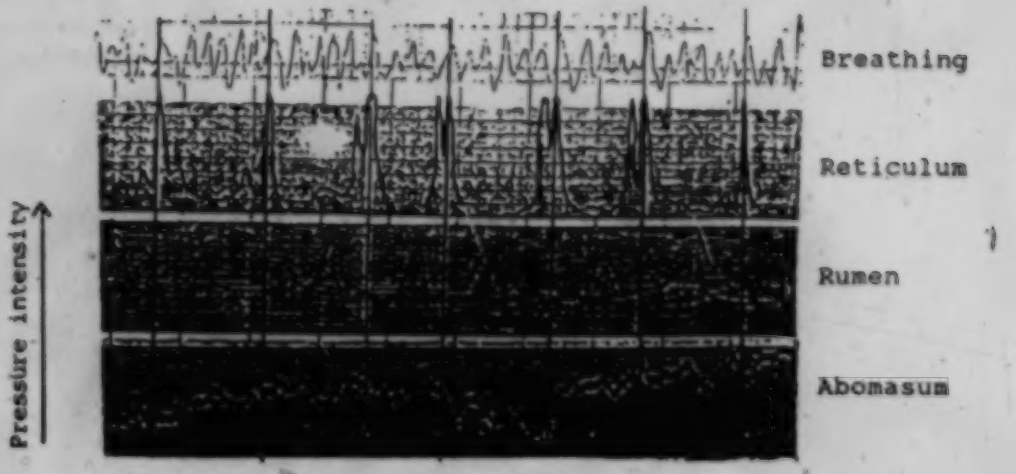


Fig. III.3: Summary of the relationship between breathing and pressure changes in the ruminant stomach

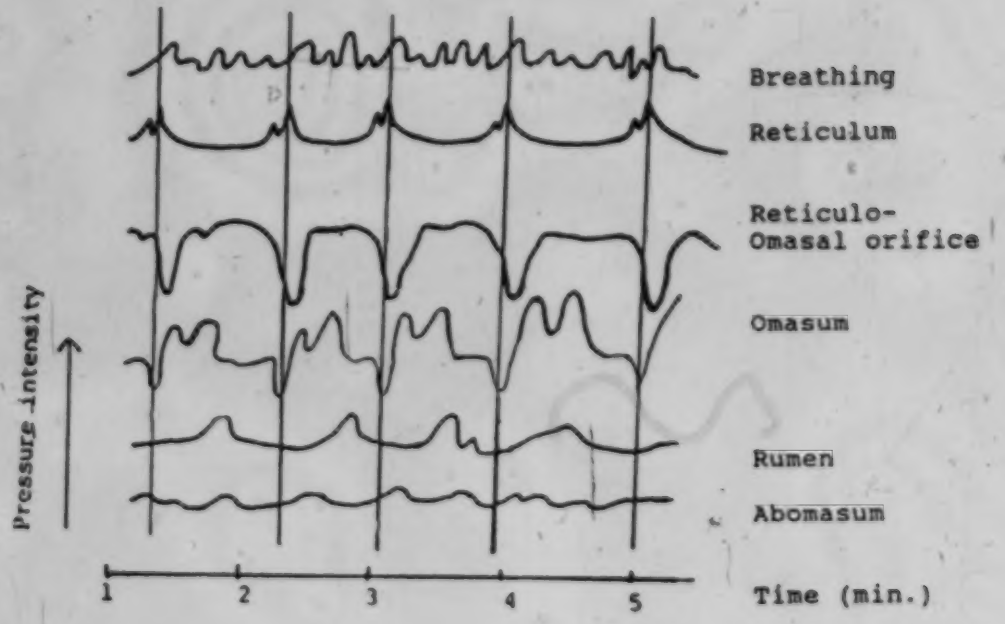
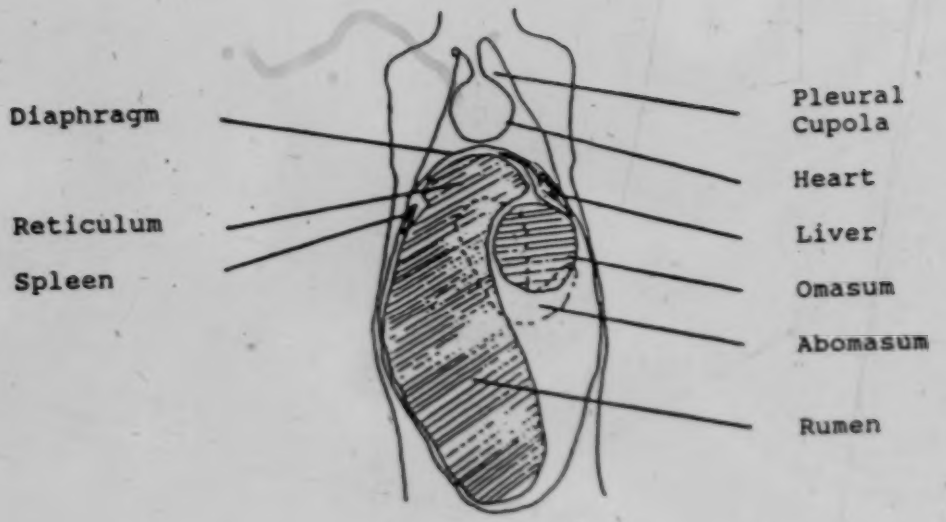


Fig. III.4: The relative positioning of ruminant viscera in schematic horizontal section.



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IV. ENDOSCOPIC TECHNIQUE TO FOLLOW THE MIXING AND BREAKDOWN OF COLORED FORAGE PARTICLES IN THE RUMINO-RETICULUM OF CATTLE.

A. INTRODUCTION

The use of re-entrant cannulae and indigestible markers has resulted in rapid advances in determining the factors influencing the rate of passage of digesta through the ruminant stomach. Recording contractions from specific locations in the rumino-reticulum has permitted the study of the sequential contractions of various regions of the stomach. However, a method yielding clear understanding of the mechanism of propulsion and movement of digesta within the stomach has not emerged. Some observations can be made directly through open fistulae but information obtained in this manner is limited. Observations have been made using radiographic techniques but the degree of resolution is quite limited. Recent advances in the technology of radiographic imaging have allowed a far more detailed study to be made of the effects of movements of various compartments, folds, pillars and orifices of the ruminant stomach on the contained digesta (Wyburn 1980). However, radiological observations of the routes taken by ingesta require the use of radio-opaque markers such as barium sulfate, which may not reveal the true pattern of the

natural particulate flow in the rumino-reticulum.

The first objective of this experiment was to find the general pattern of movement of the particulate phase in the rumino-reticulum. The second goal was to procure an idea of the breakdown rate of large particles. Finally, the last purpose was to determine the usefulness of a fibre-optics endoscope in visualizing the kinetic characteristics of the particulate rumino-reticulum phase.

B. MATERIALS AND METHODS

Three Hereford steers (400 to 409 kg body weight, 15 months of age) were prepared with rumen fistulas of 10 cm diameter. Each animal was maintained in an individual pen with continuous lighting at an ambient temperature of 20 - 22 ^{0}C during the experimental period. Brome grass hay (Bromus inermis) chopped through a 76 mm screen and harvested at mid-to-late bloom was offered at 2 h intervals (480 g dry matter, DM, each 2 hours to achieve maintenance, NRC 1984) by means of an automatic feeding device. The chemical composition of the diet was 13.9 g N kg^{-1} DM, 672 g of cell wall constituents kg^{-1}DM, 349 g acid detergent fiber (ADF) kg^{-1}DM. Cobalt iodized salt blocks and water were available ad libitum.

To acquire bromegrass stems only, the leafy material was first manually removed from 10 kg of bromegrass hay.

Half of the stems were ground in a Wiley Mill using a 2mm screen and then sieved using a wet sieving procedure (Dixon and Milligan 1985). Particles which passed through a 2mm screen but not through a 1mm screen were collected and designated small particles (SP). The other half of the stems were cut into 10 mm lengths which were designated large particles (LP). The LP and SP were washed for one hour (h) in boiling neutral detergent and then carefully rinsed with distilled water.

Small and large particles were colored separately at different times with a commercial red dye (RIT^{R}, scarlet 5, Canada Starch Co. Inc.). A metallic container was placed on a heating plate and filled with 4 liters of water. The water was heated, the dye (one envelope containing 32 g of dye) dissolved and the particles were then placed in the dyebath. While stirring, the dyebath was heated to simmering (just under boiling) for 30 minutes. The colored particles were then rinsed three times in cold tap water and dried at 40 ^{o}C for 48 h.

Fixed weights of small particles (67g) or large particles (80g) were introduced into three different rumino-reticulum locations: reticulum (location 1), rumen dorsal sac (mat, location 2), and rumen ventral sac (liquid phase, location 3). The combination of different particle sizes (two) and locations (three) produced six treatments. Five separate measurement periods were used with each particle size.

The end of the endoscope (Olympus colonoscope Model TCF-2L, Olympus Corporation of America, New Hyde Park, NY) was fitted with an adaptor made of a circular plastic window of 1.0 cm diameter with circles printed every 1 mm radius on its surface for the measurement of particle size. A 2.0 cm long tubing joined this window to the end of the endoscope. Particles were clearly visible when they were in contact with the window. This modified endoscope was used to count the number of the colored particles at seven different rumino-reticulum areas: top and bottom of the reticulum, bottom of the cranial sac, bottom of the ventral sac, top of the dorsal sac, dorsal caudal sac and ventral caudal sac.

After the introduction of the colored particles into the three different locations of the rumino-reticulum, these stained particles were counted in each area five times every h for 3 h, every 1.5 h for a further 4.5 h and every 2 h for another 4 h. A channel was made through the dense mat of rumen contents with the help of a wooden rod to permit the passage of the flexible endoscope to the observation area. The maximum number of particles for each location was obtained by plotting the summation of the small (<2 mm), intermediate (particles greater than 2 mm, but less than 10mm) and large particle numbers in the seven areas at different times.

The movement of the colored particles was followed by interpreting the appearance and change in number of

particles in each rumino-reticulum area. The breakdown of the large particles was described as the decrease in the number of the colored large particles and the increase of intermediate particles and small particles.

C. RESULTS

The small particles mixed gradually (2 to 3 h). Their number decreased, when the mixing was over, proportionally in each location (Table IV.1). The particles put in the reticulum appeared to mix slightly faster than when they were put in other locations (Table IV.1).

The LP appeared to be able to mix in all locations without distinction (Table IV.2). Consequently, the same number of LP were detected in the reticulum as the other observation areas (Table IV.3). The times needed for the LP to attain trace amount were 6.2 $\pm$ 2.6 h, 7.6 $\pm$ 2.3 h, 4.5 $\pm$ 1.5 h for the locations 1, 2, and 3, respectively. These values were not significantly different ($p>0.05$, Table IV.7). The LP were no longer detectable at 7.5 $\pm$ 1.5 h (Table IV.4).

The times at which the maximum number of colored particles of all sizes originating from the colored LP was attained were 4.5 $\pm$ 0.1 hrs, 7.5 $\pm$ 0.1 hrs, 6.5 $\pm$ 1.9 hrs when the particles were inserted in the rumen at locations 1, 2, and 3 respectively (Table 4). The time to attain the particle maximum number was shorter ($p<0.01$) after introduction of the large colored particles into the

reticulum.

The total, intermediate, and small particle numbers were at their maximums after 6.5 ± 1.7 h, 4.0 ± 0.9 h and 7.1 ± 1.9 h, respectively. The times at which the SP and the total particulate amounts were at their maximum did not differ ($p<0.05$). Conversely, there was a significant difference ($p<0.01$) between the time of the maximum count of intermediate particles versus SP and total particles maximum times (Table IV.4).

Table IV.5 illustrates the technique used to assess the movement of the small colored particles. Figure IV.1, which was constructed to represent the increases and decreases of the colored particles, indicates that most of the movement of small particles was from the anterior to the posterior of the rumen in the lower layer and then from the caudal sac to the anterior by way of the dorsal layer of the rumen digesta. However, a back flow of small particles through the mat is possible.

As with the small particles, the movement of the large particles was circular originating at the anterior, moving back in the ventral layer of the rumen digesta and returning to the anterior of the rumen through the dorsal layers. A back flow of large particles from the front to the back in the surface digesta layer was less common (Fig. IV.2). A example of data used to construct the figures is given in Table IV.2.

Figure IV.3 indicates the general movement of the

particulate matter in the rumino-reticulum. This movement is circular, initiating in the front and moving back via the bottom of the rumen and moving from the back to the front in the top of the rumen.

D. DISCUSSION

It appeared that when the large particles were placed in the reticulum, the time needed to obtain the maximum peak of particles of all sizes was significantly smaller ($p<0.01$) than with other places of introduction. A possible explanation could be that the large particles introduced at this location were subjected to chewing during rumination sooner than those from the rumen.

The number of large particles decreased relatively quickly. The time needed to attain trace amount of LP was not significantly affected by the place of introduction. Most of the large particles were reduced in size (<10 mm) after only an average of 7.0 $\pm$ 1.5 h (Table IV.4). This is an indication of the efficiency of the particle size reduction processes. This is more rapid than the approximately 25 hours required to reduce long particles (10 mm) of NDF-extracted ^{51}Cr mordanted brome stems to smaller than a specific size (3.35 mm) in the previous experiment of this thesis.

The times at which the total number of particles and the small particles number were at their maximum, and the large particle number were only minimal coincided at about

7.0 h. This can be expected since an increase in the total number of particles means a significant decrease of the original large particles and an increase of their broken parts in the intermediate (IP) and small particles. The IP attained their maximum level before the SP ones which would be expected since SP in the rumen would originate in part from the IP.

The disappearance of the small particles after mixing was uniform in all seven rumino-reticulum areas. It appeared that the rumino-reticulum acted as a true pool of semi-liquid content in which particulate matter introduced inside at different locations mixed completely after a short period of time with no obvious barriers (Table IV.1).

The pattern of movement of the small and large particles was similar. One important difference is that the small particles apparently can travel through the mat in the opposite direction to the principal particulate flow. This phenomenon may help small particles to mix faster. The general movement seems to indicate that the new particles, originating from the bolus, would pass under the compact mat floating on the surface and go to the posterior part of the rumen. This new particulate matter would then float in the dorsal sac coming from the posterior part of the rumen to the anterior rumino-reticulum portion. The older particulate material, which is still floating, will go first to the reticulum to be ruminated. Thus, the new material would have to wait for the breakdown of the old,

and probably would be partially fermented during this time. The general movement appears to be a relatively simple circular stream inside the rumino-reticulum, with some backflows and mixing actions possible.

Large particles as well as small particles were mixed uniformly inside the rumino-reticulum after a few hours. These results question the notion of a hypothetical barrier (occlusion of coarse matter in the floating mat of fiber, Van Soest 1982) limiting the flow of large particles to the reticulum (Tables IV.2 and IV.3).

These results also do not totally agree with Waghorn and Reid (1977) or Wyburn (1980). These authors stated that the kinetics inside the rumino-reticulum are divided in two streams; a circular stream from the anterior to the posterior of the rumen at the lower level of the mat, followed by a movement from the posterior to the anterior on the top of the mat in the dorsal sac, and a circular stream which was in the opposite direction in the ventral sac. It appears that our results indicated only the movement that these authors described in the dorsal sac. Their experiments involved sheep, so it is possible that the kinetics of the particulate material inside the rumino-reticulum of cattle and sheep are different. Their experiments also involved radiological techniques with the use of liquid radio-opaque subtances, which can give only the liquid phase movement of the digesta.

It is concluded that the endoscopic technique is useful

to study the distribution pattern of particulate matter as well as its breakdown.

TABLE IV.1. Count of the colored small particles in the seven areas ofobservation at different times after introduction into the rumino-reticulum at the three locations (average of the three steers).

TIME (h)	NUMBER OF PARTICLES																								
	LOCATIONS[e]																								
	1								2								3								
	AREAS[f]								AREAS								AREAS								
	1	2	3	4	5	6	7	T	1	2	3	4	5	6	7	T	1	2	3	4	5	6	7	T
0	17	34	26	12	0	0	13	102	0	0	1	0	1	0	95	87	2	34	31	0	0	0	40	157
1.0	13	10	4	2	21	14	10	84	59	25	7	53	2	8	7	164	11	17	6	10	10	6	11	71
2.0	10	12	7	12	11	18	11	81	19	52	45	35	19	25	37	232	4	7	5	11	17	7	6	57
3.0	3	4	7	6	13	15	13	61	26	16	41	26	10	22	33	174	6	7	9	5	14	10	4	55
4.5	14	7	6	8	7	11	10	69	24	16	32	30	40	19	15	176	7	8	8	7	6	12	6	48

[e]LOCATIONS: Sites of addition of the colored particles.
1: Reticulum.
2: Dorsal sac.
3: Ventral sac.

[f]AREAS: Sites where the colored particles were counted.
1: Top of the reticulum.
2: Bottom of the reticulum.
3: Bottom of the cranial sac.
4: Bottom of the ventral sac.
5: Ventral caudal sac.
6: Dorsal caudal sac.
7: Top dorsal sac.
T: Total of the particles in the seven areas.

TABLE IV.2. Technique used to assess the movement of the large particles and their rate of breakdown. Here the large colored particles were placed in location 2 (mat, dorsal sac).

TIME (h)	NUMBER OF COLORED PARTICLES																													
	ALL SIZES							SPECIFIC SIZES[θ]																						
								LARGE							INTERMEDIATE							SMALL						
	AREAS[ƒ]							AREAS							AREAS							AREAS						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
0	2	0	1	0	5	6	45	1	0	0	0	0	0	42	0	0	0	0	0	0	3	1	0	1	0	0	0	0
1.50	28	1	0	1	0	0	6	19	1	0	0	0	0	4	9	0	0	0	0	0	2	0	0	0	1	0	0	0
3.00	11	4	9	1	0	0	5	5	0	4	1	0	0	0	6	4	5	0	0	0	4	0	0	0	0	0	0	1
4.50	5	1	3	9	4	1	5	0	0	0	1	1	0	1	1	1	3	6	2	0	3	4	0	0	2	1	1	1
6.0	8	8	1	1	9	3	4	1	0	0	0	1	0	0	2	5	1	1	5	3	1	5	3	0	0	3	0	3

[θ]SIZES:
LARGE: 10mm.
INTERMEDIATE: particles greater than 2 mm, but smaller than 10 mm.
SMALL: ≤2 mm.

[ƒ]AREAS: Sites where the particles were counted.
1 : Top reticulum.
2 : Bottom reticulum.
3 : Bottom of the cranial sac.
4 : Bottom of the ventral sac.
5 : Ventral caudal sac.

TABLE IV.3. Total numbers of colored particles of all sizes (small, intermediate and large) after introduction of the large colored particles into the 3 rumino-reticulum locations.

TIME (h)	NUMBER OF PARTICLES																							
	LOCATIONS[1]																							
	1								2								3							
	AREAS[2]								AREAS								AREAS							
	1	2	3	4	5	6	7	T	1	2	3	4	5	6	7	T	1	2	3	4	5	6	7	T
0	0	17	2	7	6	0	3	35	1	0	1	1	0	0	45	48	0	3	19	0	0	1	2	25
1.5	0	3	3	6	1	6	1	20	28	1	0	1	0	0	6	36	2	7	5	11	14	0	0	39
3.0	0	2	10	8	6	5	4	35	11	4	9	1	0	0	5	30	3	7	12	7	7	2	3	41
4.5	3	4	9	10	9	7	6	48	5	1	3	9	4	1	5	28	6	10	11	6	4	1	5	44
6.0	8	3	5	5	10	8	3	42	8	8	1	1	9	3	4	34	2	7	10	7	10	5	5	46
7.5	4	7	8	5	6	4	7	41	10	11	6	9	11	3	9	58	5	7	8	10	8	9	5	53

[1] Locations: Sites of addition of the colored particles.
1: Reticulum
2: Dorsal sac.
3: Ventral sac.

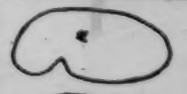
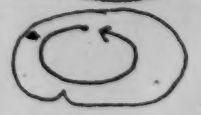
[2] Areas: Sites where the colored particles were counted.
1: Top of the reticulum.
2: Bottom of the reticulum.
3: Bottom of the cranial sac.
4: Bottom of the ventral sac.
5: Ventral caudal sac.
6: Dorsal caudal sac.

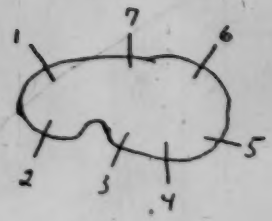
Table IV.4. Time (h) when the total, intermediate and small particle numbers were at their maximum and the large particle numbers at minimum after the addition of large particles at locations 1, 2, 3.

	LOCATION 1	2	3	$\bar{X}$+S.D.*	Locations 1+2+3**
Max. Total	4.5±0.1	7.5±0.1	7.5±1.9	6.1±0.5b	7.5
Max. Inter.	4.5±1.0	3.0±1.2	4.5±1.4	4.0±0.9a	4.5
Max. Small	4.5±1.1	7.5±1.3	7.5±2.0	7.1±1.9b	7.5
Min. Large	6.2±2.6	7.6±2.3	4.5±1.5	7.0±1.5b	7.5

* arithmetic mean
** graphically obtained
a, b: Values in the same column followed by the same letter are not significantly different ($p < 0.01$). (Duncan Multiple Range Test)
Max.: Maximum.
Min.: Minimum.
Location: Site of addition of the colored particles into the rumino-reticulum.
1 : Reticulum
2 : Rumen dorsal sac.
3 : Rumen ventral sac.

TABLE IV.5. Technique used to assess the movement of the small colored particles. Here, with particles placed originally in location 2.

TIME (h)	NUMBER OF SMALL COLORED PARTICLES								DIAGRAM
	AREAS[@]							TOTAL	
	1	2	3	4	5	6	7	Anterior Posterior	
0	0	0	1	0	1	0	95	97	
1	59	25	7	53	2	8	7	164	
2	19	52	45	35	19	25	37	232	
3	26	16	41	26	10	22	33	174	
4.5	24	16	32	30	40	19	15	176	



[@]AREAS: Sites where the particles were counted.
1: top of the reticulum.
2: bottom of the reticulum.
3: bottom of the cranial sac.
4: bottom of the ventral sac.
5: ventral caudal sac.
6: dorsal caudal sac.
7: top of the dorsal sac.

Figure IV.1. Movements of the small particles in the rumino-reticulum.

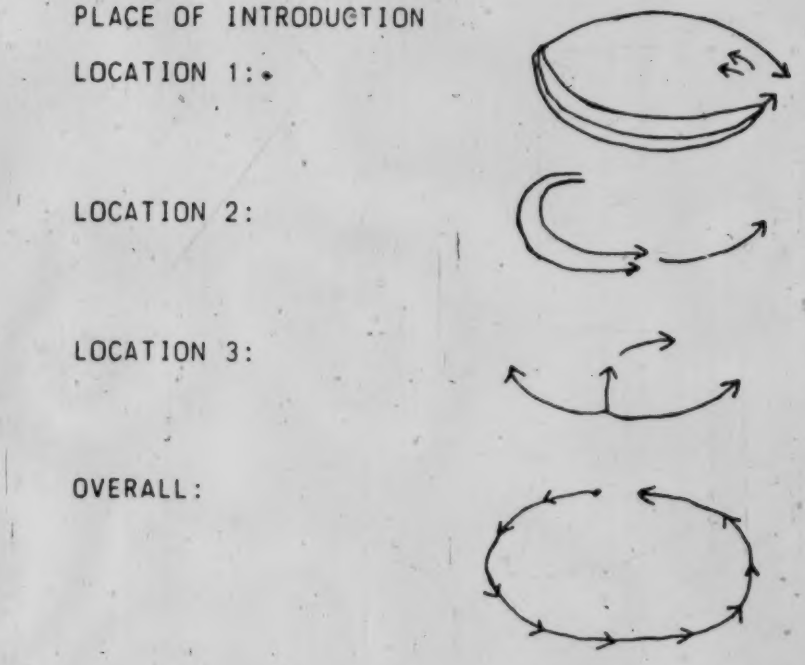


Figure VI.2. Movement of the large particles in the rumino-reticulum.

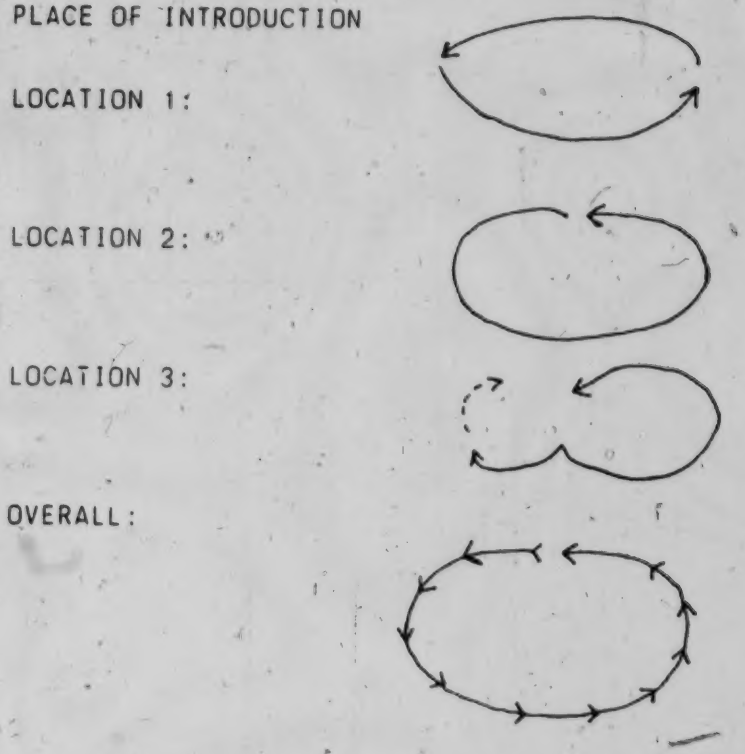
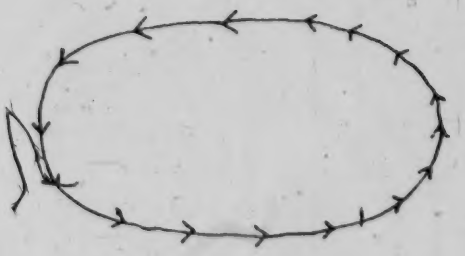


Figure VI.3. General movement of large and small particles.



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V. BUOYANCY SEPARATION OF PARTICLES IN FEED, FECES, AND RUMEN CONTENTS AND THEIR EXAMINATION BY NUCLEAR MAGNETIC RESONANCE.

A. INTRODUCTION

Specific gravity affects passage of inert particles (King and Moore 1957; Evans et al. 1973; desBordes 1984) and chromium-mordanted alfalfa (Ehle 1984) from the rumen. Particles with specific gravity between 1.17 and 1.42 (desBordes 1984) sank through the fibrous mat in the rumen and were more likely to be passed through the reticulo-omasal orifice than were particles outside this range. Thus, this particle property is likely to influence voluntary consumption in forage-fed ruminants.

The nutritive value of feeds and forages is greatly affected by physical form and other physical attributes (Van Soest 1982). It is probable that the shape and nature of the particles are related to their chemical composition. Ehle and Stern (1984) stated that the distinction between chemical and physical characteristics of feedstuffs is usually vague, since chemical composition often ultimately determines the physical attributes of feed (e.g., density and particle shape).

The present practice of describing forage cell wall constituents as a broad category including cellulose,

hemicellulose and lignin is crude and may mask significant underlying physical and chemical differences. Further research in this field is needed. Progress may first require utilization of more refined analytical techniques. Nuclear magnetic resonance (N.M.R.) is an example of such a technique (Ehle and Stern 1984). Preliminary studies of the nutritional value of forages by ^{13}C cross-polarization-magic angle spinning with proton decoupling NMR indicate that protein, lignin and carbohydrate can be seen on spectra directly and may be estimated at least semi-quantitatively (Elofson et al. 1984).

The objectives of this research were to investigate the separation of particles from feed, feces and rumen on the basis of their buoyancy, to examine the dimensions and shape of these particles, and to study their composition by high resolution solid state ^{13}C-NMR.

B. MATERIAL AND METHODS

One 15-month old Hereford steer (405 kg) was prepared with a rumen fistula (10 cm diameter). The animal was maintained in an individual pen with continuous lighting at an ambient temperature of 20-22 ^{o}C during the experimental period. Bromegrass (Bromus inermis, mid-to late-bloom) hay, chopped through a 76 mm screen was offered at a maintenance feeding level (480g dry matter, DM, every

2 hours, NRC 1984) by means of an automatic feeding device. The diet contained 11.5% crude protein (CP), 672 g cell wall constituents (neutral detergent fiber, NDF), and 349 g acid detergent fiber (ADF) per kg DM. Cobalt mineralized salt blocks and water were available ad libitum.

To obtain feed particles, bromegrass hay stems were ground in a Wiley Mill using a 2 mm screen and then sieved by a wet sieving procedure that allowed the particles to pass through a 2 mm screen but not through a 1 mm screen (Dixon and Milligan 1985). The resulting particles were then soaked in physiological saline for 24 hours.

The feces were also wet-sieved using a 0.5 mm screen to isolate the particulate matter. After drying the particles were ground in a Wiley Mill using a 2 mm screen and then sieved with the previously described wet sieving technique. The >1 <2 mm particles were then dried at 60 ^{0}C in a force draft oven for 48 hours. Prior to addition to the fluid in the cylinder, they were soaked in physiological saline for 24 hours.

Three types of rumen particles were studied. Two were isolated from the mat on the rumen contents in the dorsal sac of the rumen and the third from the ventral sac. The material originating from the mat was wet-sieved into two fractions; a small particle (<3.35 mm) and a larger particle (>3.35 mm) fraction. These fractions were dried and ground separately in a Wiley Mill (2 mm screen). The resulting particles were then treated similarly to the feed

particles. The small particles (<3.35 mm) originating from the ventral sac received the same treatment as the small particles from the mat.

Rumen contents were filtered through eight layers of cheesecloth. A cylinder (114.3 cm long and 9.0 cm in diameter, graduated every 100 mL) was filled with 4.5 L of rumen fluid. To maintain an anaerobic environment, a CO_2 atmosphere was maintained at the top of the cylinder. The cylinder was held in a water bath at 39 ^{0}C. After 0.5 hour, a green flocculation was apparent on the top of the liquid in the cylinder. This was removed by suction and the volume was adjusted to 4.0 L.

Soaked particulate matter (5 g DM) was put on the top of the rumen fluid in the cylinder. The liquid in the cylinder was emptied 10 seconds after deposition of the particles on the top, through a small opening (8 mm) at the bottom with 200 mL portions being collected in twenty separate containers. The cylinder was emptied with the bottom opening totally unrestricted. The different fractions were numbered one to twenty, twenty being from the top and one from the bottom of the cylinder. When the cylinder was finally empty, the contents of the different containers were washed with tap water using a cylinder with a 0.1 mm screen to clean soluble components off the particles. The particles were then dried in a forced draft oven at 60 ^{0}C for at least 48 h and subsequently weighed.

The shape and dimensions of the particles separated by

buoyancy were evaluated using a microscope and a high precision ruler.

The particles from each 200 mL fraction were examined by high resolution solid state ^{13}C-NMR. Carbon-13 NMR spectra were obtained on a Bruker CXP-200 spectrometer at a frequency of 50.3 MHZ. A single, matched cross-polarization contact of 2 msec was used; 8000 free induction decays were collected over 1K memory and zerofilled to 8K before Fourier transformation. Magic angle spinning rate was 4.0 KHZ (Elofson et al. 1984).

N content of the samples subjected to NMR spectroscopy was determined by the standard Kjeldahl method (Association of Official Analytical Chemists 1985). The specific gravity of the filtered rumen fluid was determined by weighing 500 mL of this liquid in a volumetric flask. The procedure was repeated five times and the average was calculated. The specific gravity of the fluid before suction of the green floating substance was 1.0069 $\pm$ 0.0002 and after was 1.0077 $\pm$ 0.0002.

C. RESULTS AND DISCUSSION

Buoyancy curves and NMR examination

The distribution curve of feed particles in the cylinder (Fig. V.1) was hyperbolic with a smaller slope for the descending phase. The maximum concentrations of particles were found at level 6 - 7. A relatively large

proportion (9.2±2.8%) of feed particles floated in fraction 20 (Fig. V.1). The maximum for fecal particles was at the level of 1 - 2; few (2.7%) fecal particles floated on the rumen liquid in the cylinder (Fig. V.2).

The shape of the distribution curve of the rumen particles contained in the liquid phase was hyperbolic (Fig. V.3). Maximum concentration of rumen particles was at the level 4-6 (Fig. V.3) and small particles in the mat (Fig. V.4) peaked at level 5. The descending portions of the two curves were very steep, particularly for the small particles originating from the liquid fraction of the rumen indicating a small proportion of particles of low density. The distribution curve of large particles from the mat (Fig. V.5) was not as simple as the other curves; in fact a major portion of the particles (41%) floated.

NMR spectra of particulate matter of all studied origins indicated that particles which floated contained a higher proportion of proteins, as well as a lower proportion of lignin (Fig. V.7, V.9). On the other hand particles which sank faster contained a higher proportion of lignin and a lower proportion of proteins (Fig. V.6, V.8).

NMR spectra of feed particles indicated an intermediate content of protein and lignin. Concerning the rumen particles, the large particles from the mat appeared to contain most of the nutritive value. More than 40% of the particles originating from the large component of the mat

floated. They contained a larger proportion of proteins (Fig. V.10). On the other hand the small particles from the mat and the liquid phase appeared to be intermediate and contained a relatively lower proportion of proteins and a larger proportion of lignin than the large particles. These observations indicated a lower nutritive value. This is in agreement with Pond et al. (1984) who stated that simple reduction in particle size does not mean the material will be more digestible, or that the rate of digestion will be greater. Akin and Burdick (1981) found that many small particles were deeply stained with acid phloroglucinol, indicating their high content of lignin and low digestibility (Akin and Burdick 1981). No increase in digestibility would be predicted from further particle size reduction or increased surface area exposure. The fecal separation curve and the analysis of NMR spectrum indicated that the fecal particles contained a low concentration of proteins and a relatively large amount of lignin.

Chemical analysis of the N content of the different stratified layers supported the NMR indication of a high content of proteins in the particulate matter having an higher buoyancy (Table V.1).

Shapes and dimensions of the particles separated by buoyancy

The analysis of the shape and dimensions of the particles separated by buoyancy (Table V.1) indicated that

the particles which sank faster, tended to be slightly longer, wider and thicker. The shape of these particles was also relatively more cubic. The particles that floated were relatively thin. The same observation was valid for all samples: faeces, feed, liquid phase particles, and large and small particles from the mat. These observations of shape relate to the reports of Milchunas (1978) and Van Soest (1982) who stated that plant tissue with a low lignin-cellulose ratio will tend to bend rather than break, while tissue with a high lignin-cellulose ratio will tend to break rather than bend. Consequently, the less lignified grasses tend to mill into long, thin fibrous particles while more lignified alfalfa fragments into more cuboid pieces. Lignification increases the force required to shear such that in milling more lignin becomes selectively distributed among the larger particles. These statements re confirmed by the NMR analysis.

All the samples were mechanically ground using the same screen in the grinder. However, feed and large particulate matter from the mat received a more rigorous mechanical grinding treatment due to their original size. Unfortuately, the effect of a stronger mechanical grinding treatment on particle shape distribution is not known.

The particles, which sank faster, are important because of their high content in lignin and their likely corresponding low digestibility. A negative association of digestibility with lignin or fiber content is well

established (Van Soest 1965). Lignin will limit the in vitro extent of degradation (Cross et al. 1974) and reduce the availability of structural carbohydrates. Ehle and Stern (1984) indicated that lignin inhibited digestion of 1.4 times its weight of cell wall carbohydrates. Smith et al. (1972) have shown that 75% of the variation of cell wall digestibility can be attributed to lignification.

Plant cell walls are not of uniform density; mature lignified walls are much denser than young immature ones, yet both are consumed to a relatively equal extent (Van Soest 1982). Consequently, both forage density and plant cell wall density have lower correlations with voluntary intake than does cell wall content (Van Soest 1982). The explanation for the failure of density to account for cell wall intake is not well understood (Van Soest 1982). The present experiment might give an explanation. Clearance depends on two interacting processes: the first being breakdown through the physical reduction in particle size plus microbial digestion, and the second being physical passage from the rumino-reticulum (Faichney 1986). Immature, voluminous, thin-walled cells are not only more digestible but have an higher buoyancy. On the other hand, the mature lignified cell walls have an higher density and a lower buoyancy. They will tend to sink through the fibrous mat in the rumen and to be more likely to pass through the reticulo-omasal orifice. Clearance of feed residues from the rumino-reticulum has long been recognized

as a major process determining and, therefore, controlling both the intake and digestion of forages (Ulyatt et al. 1986). Thus, young immature and highly digestible cell walls will disappear from the rumino-reticulum mainly by microbial digestion while mature lignified cell walls will exit mainly by passage through the reticulo-omasal orifice.

It appeared that the relative buoyancy of the particles is based simultaneously on their chemical and physical properties. Physical form and attributes are probably largely determined by the chemical composition. Buoyancy might be related directly to the shape and chemical composition or only directly to the physical form and indirectly to the chemical composition. The thinner particles might sink slower because of their hydrodynamic properties, as for example as a feather in the air, or/and also because of the density of their chemical components. Unfortunately there is no information in the literature on these relationships. It appeared that no research has been done on relative densities of proteins versus fibers. The interrelationship between buoyancy, specific gravity, shape and dimensions, and chemical composition of particulate matter might be useful in an indirect analysis of the nutritive value of forage particles by relative buoyancy.

Table V.1: Protein content, dimensions and shape of isolated particles

Material	Level*	C.P.@ (%)	Length (mm)	Width (mm)	Thickness (mm)	Shape: Cuboid a	Rectangular b§	Rectangular c-	Fibrous d'	Fibrous e//
Feces										
	17	3.17	2.17±0.75	0.42±0.45	0.20±0.10	>	>>	>>	>	0
	20	4.52	1.71±1.05	0.23±0.29	0.12±0.04	0	0	>	>>>	>
Feed										
	1	5.06	2.63±0.74	0.63±0.61	0.26±0.05	>	>>	>	0	0
	7	6.03	2.50±0.76	0.66±0.65	0.23±0.05	0	>	>>	>	0
	20	8.88	2.36±0.75	0.76±0.65	0.18±0.08	0	>	>	>>	>
Liquid phase particles										
	1	4.06	2.06±0.53	1.07±0.67	0.38±0.14	>	>	>	0	0
	20	6.24	1.40±0.74	0.35±0.37	0.15±0.14	0	0	0	>	>>>
Large particles from mat										
	1	2.85	1.92±0.67	0.89±0.58	0.32±0.04	0	>>	>>	>	0
	20	4.67	1.13±0.15	0.32±0.16	0.12±0.04	0	0	>	>>	>>
Small particles from mat										
	1	1.99	1.78±0.67	1.38±0.52	0.38±0.15	>>	>	>	0	0
	5	3.23	2.75±0.42	0.92±0.20	0.28±0.15	0	>>>	>	0	0
	17	6.60	2.00±1.11	0.13±0.06	0.14±0.05	0	0	>	>>>	>>
	20	7.04	1.78±0.83	0.07±0.04	0.07±0.04	0	0	0	>	>>>

*Level: represents consecutive 200 mL portions from the bottom (1) to the top (20) of the cylinder.

@C.P.: crude protein.

"a: cuboid shape particles; length = 1.4 to 1.0 mm; width = 1.4 to 0.8 mm; thickness: 0.5 to 0.3 mm.

§b: large rectangular shape particles; length = 2.8 to 2.0 mm; width = 1.0 to 0.7 mm; thickness = 0.4 to 0.30 mm.

-c: small rectangular shape particles; length = 2.0 to 1.4 mm; width = 0.7 to 0.4 mm; thickness = 0.3 to 0.2 mm.

'd: large fibrous shape particles; length = 27 to 1.4 mm; width = 0.5 to 0.2 mm; thickness = 0.2 to 0.1 mm.

//e: small fibrous shape particles; length = 1.4 to 0.7 mm; width = <0.1 mm; thickness = <0.1 mm.

0: very small proportion of the particles (0-5%).

>: 10-15% of the particles.

>>: 20-30% of the particles.

>>>: 40-50% of the particles.

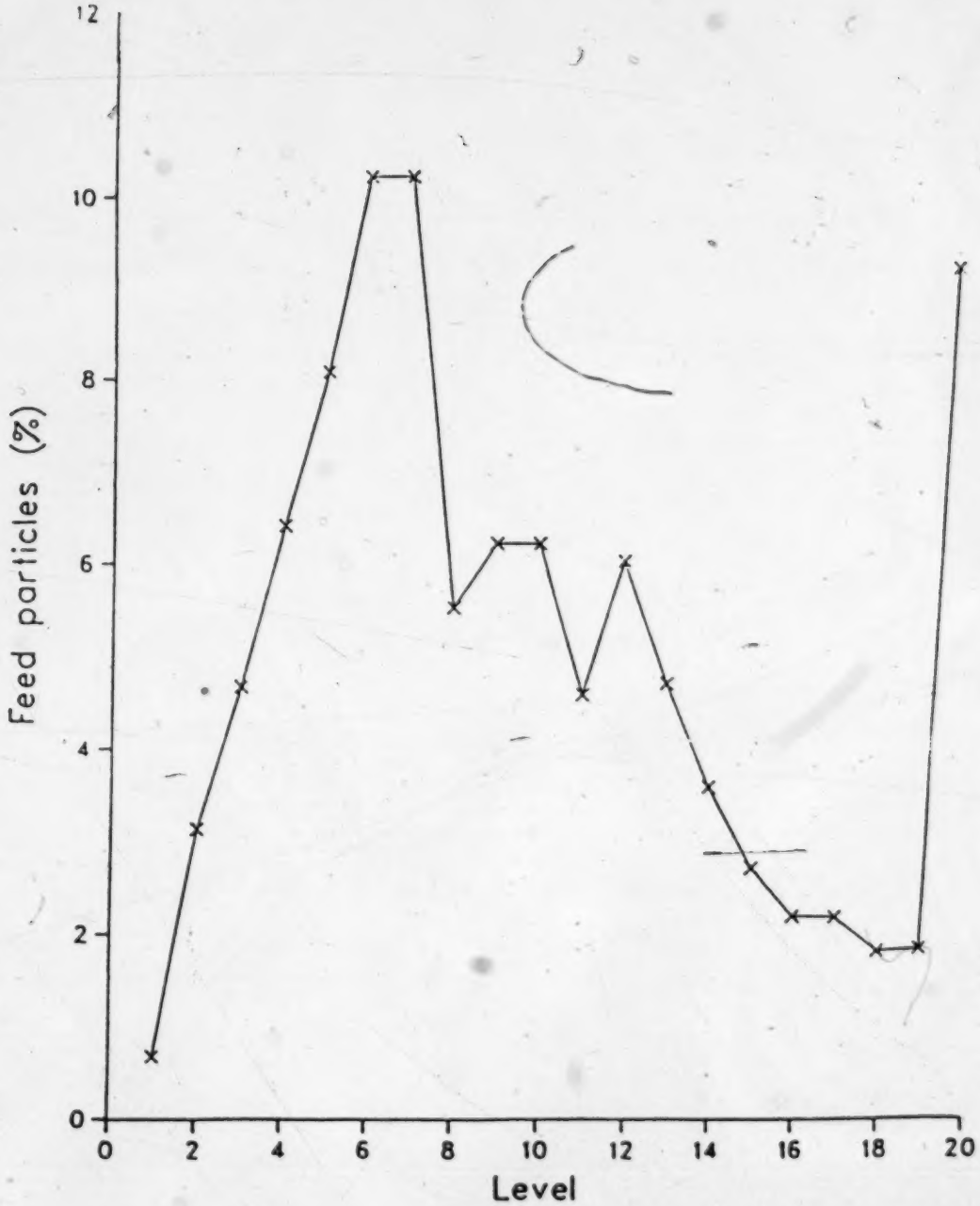

Fig. V.1. Percentage of ground (1-2 mm) feed particles in each level of the cylinder. Level represents consecutive 200 mL portions from the bottom (1) to the top (20) of the cylinder.

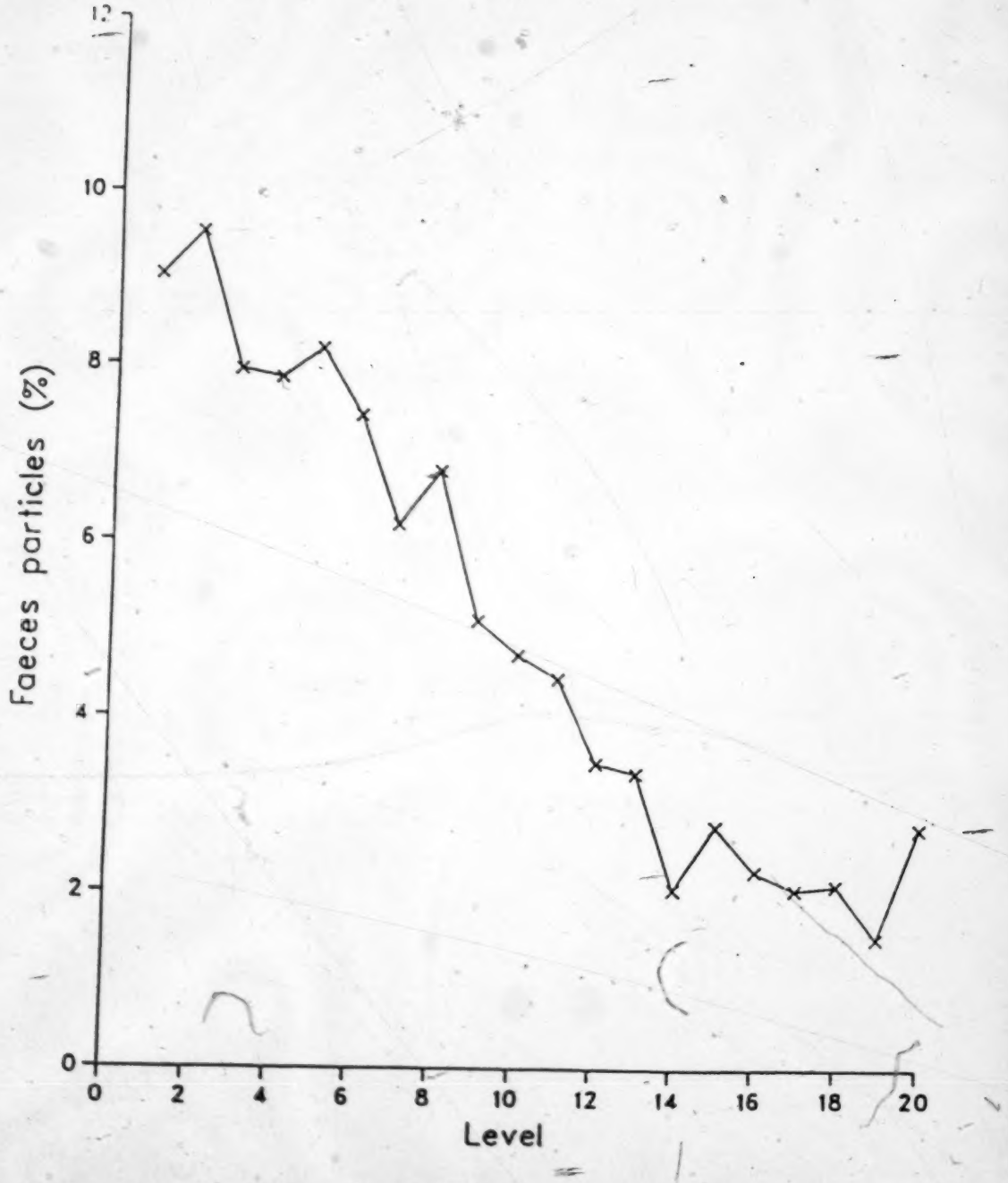

Fig. V.2. Percentage of ground (1-2 mm) feces particles in each level of the cylinder. Level represents consecutive 200 mL portions from the bottom (1) to the top (20) of the cylinder.

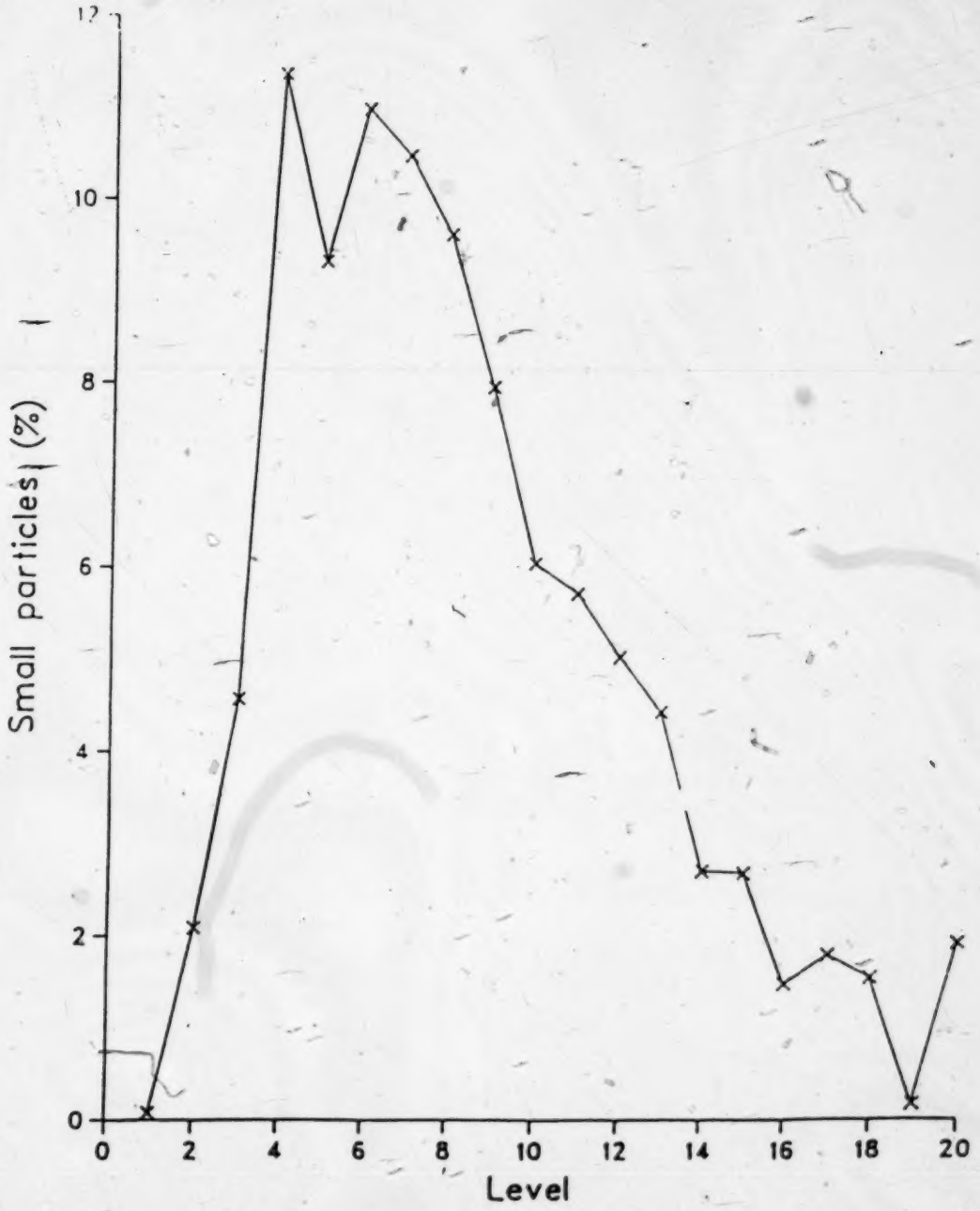

Fig. V.3. Percentage of ground (1-2 mm) small particles in the liquid fraction of the rumen in each level of the cylinder. Level represents consecutive 200 mL portions from the bottom (1) to the top (20) of the cylinder.

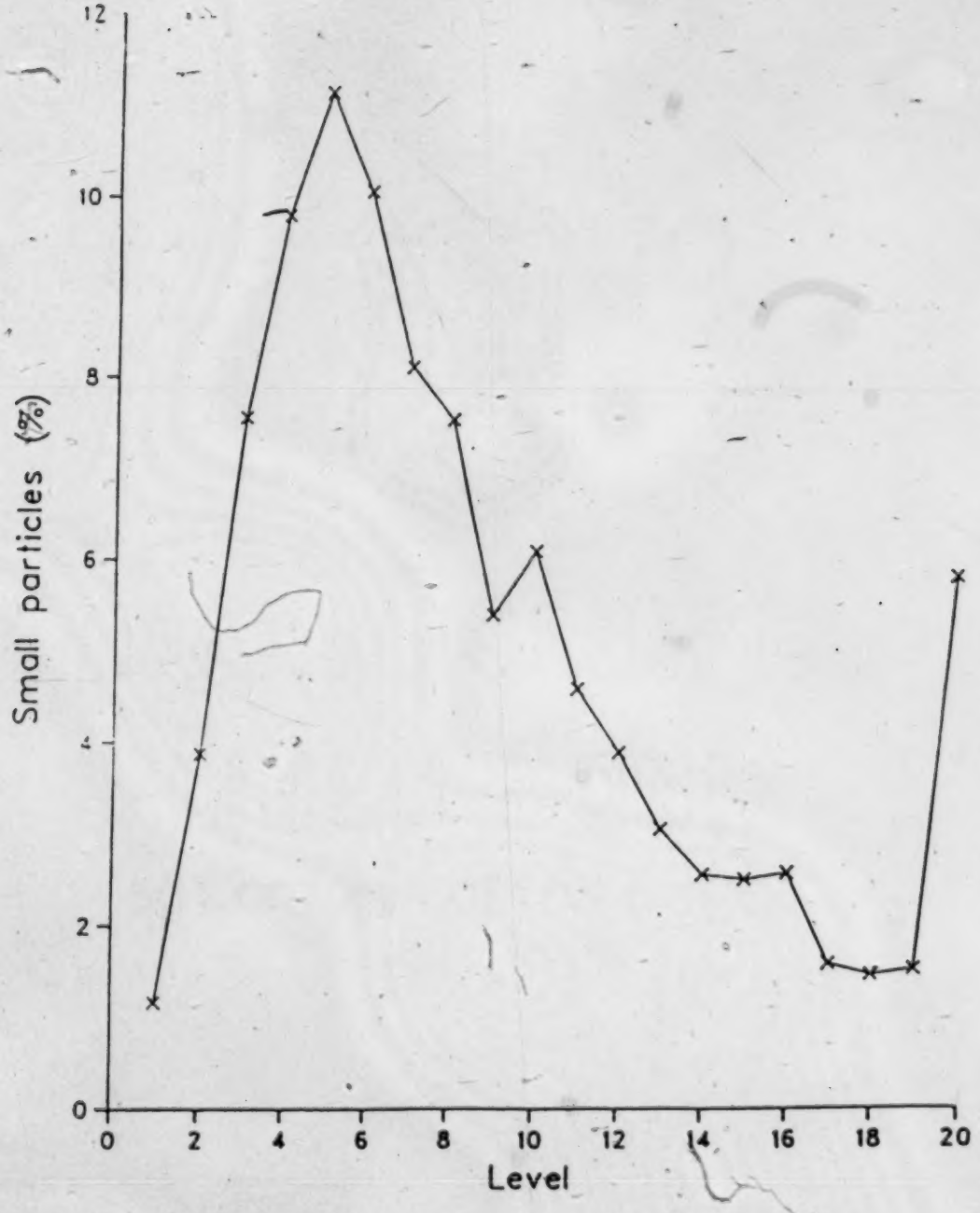

Fig. V.4. Percentage of ground (1-2 mm) small particles from the mat in each level of the cylinder. Level represents consecutive 200 mL portions from the bottom (1) to the top (20) of the cylinder.

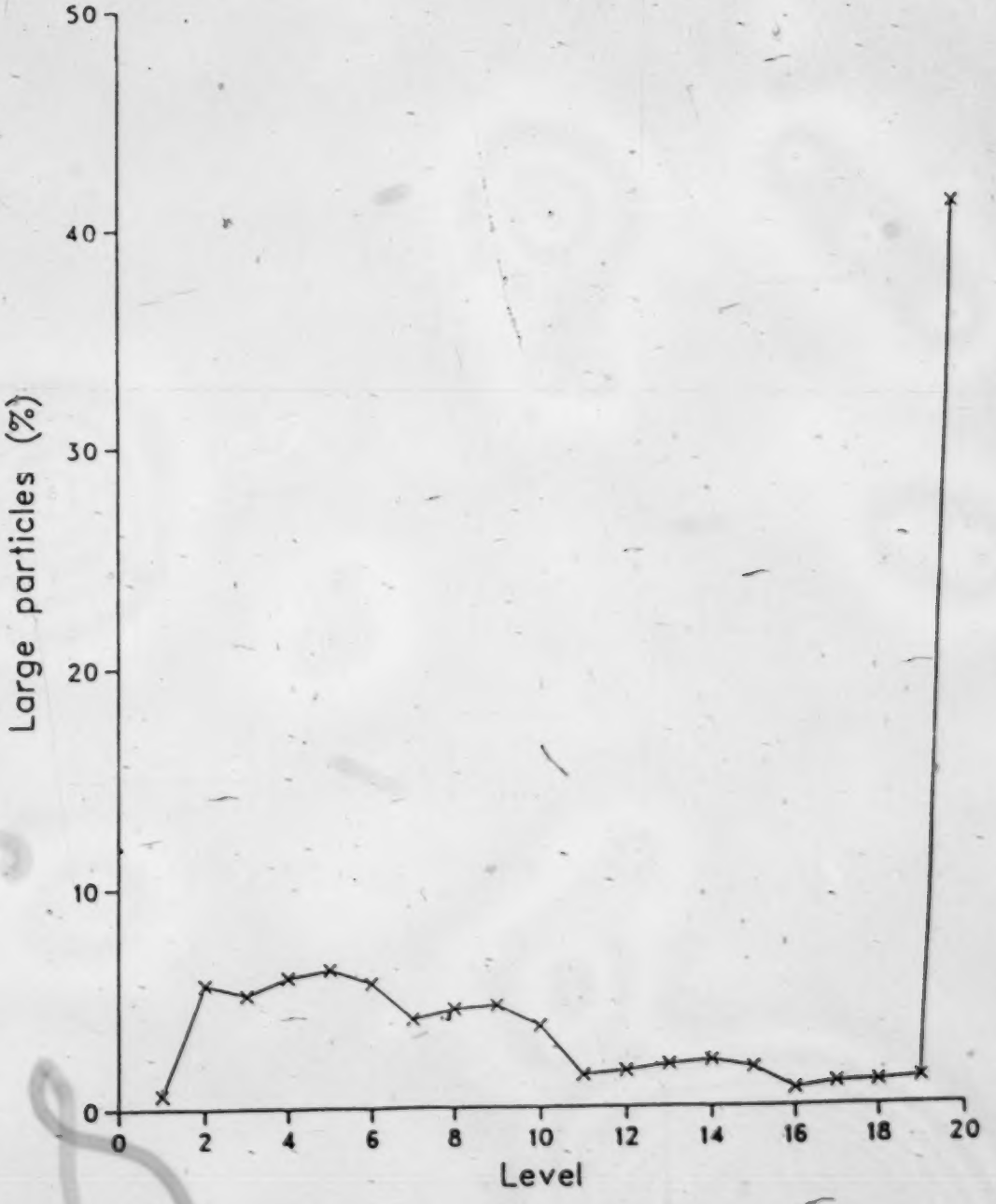

Fig. V.5. Percentage of ground (1-2 mm) large rumen particles in each level of the cylinder. Level represents consecutive 200 mL portions from the bottom (1) to the top (20) of the cylinder.

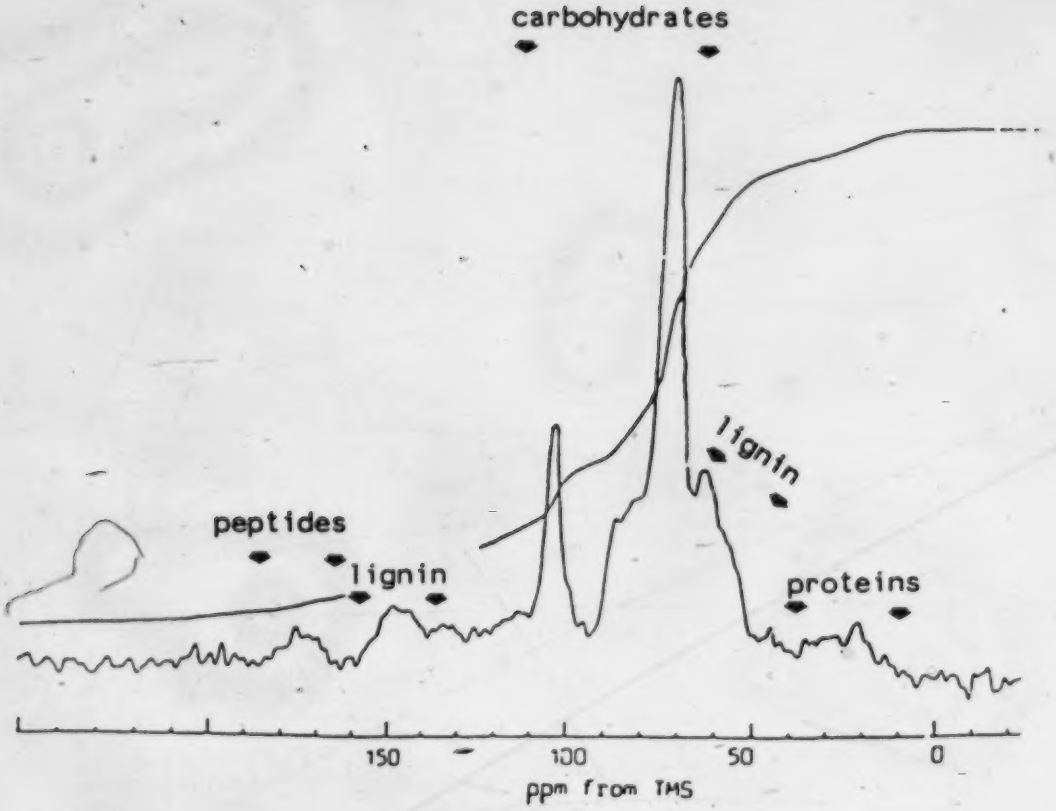

Fig. V.6. NMR spectrum of low buoyancy feed particles. The abcissa in this spectrum represents chemical shifts given in parts per million from the carbon of tetramethylsilane. This scale represents the magnetic shielding due to the electrons of chemically distinct carbon nuclei. On this spectral scale, carbonyl carbons associated with peptide linkages, and with hemicellulose appear at 170-180 ppm. The aromatic carbons, chiefly of lignin, appear at 130-160 ppm. Between 60 and 105 ppm oxygenated aliphatic carbon atoms associated chiefly with carbohydrate appear. The peak at 56 ppm is of the carbon of methoxyl groups, chiefly of lignin. At 5-50 ppm, aliphatic carbons appear that have been attributed largely to protein.

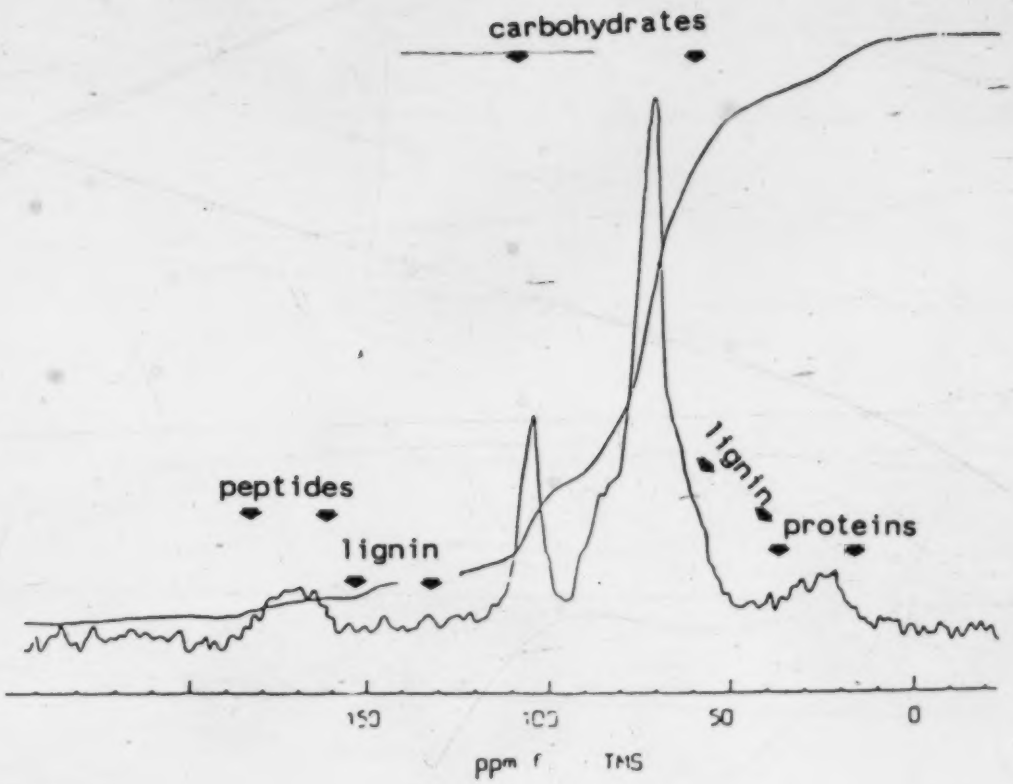

Fig. V.7. NMR spectrum of high buoyancy feed particles. The abcissa in this spectrum represents chemical shifts given in parts per million from the carbon of tetramethylsilane. This scale represents the magnetic shielding due to the electrons of chemically distinct carbon nuclei. On this spectral scale, carbonyl carbons associated with peptide linkages, and with ester groups associated chiefly with hemicellulose appear at 170-180 ppm. The aromatic carbons, chiefly of lignin, appear at 130-160 ppm. Between 60 and 105 ppm oxygenated aliphatic carbon atoms associated chiefly with carbohydrates appear. The peak at 56 ppm is of the carbon of methoxyl groups, chiefly of lignin. At 5-50 ppm, aliphatic carbons appear that have been attributed largely to protein.

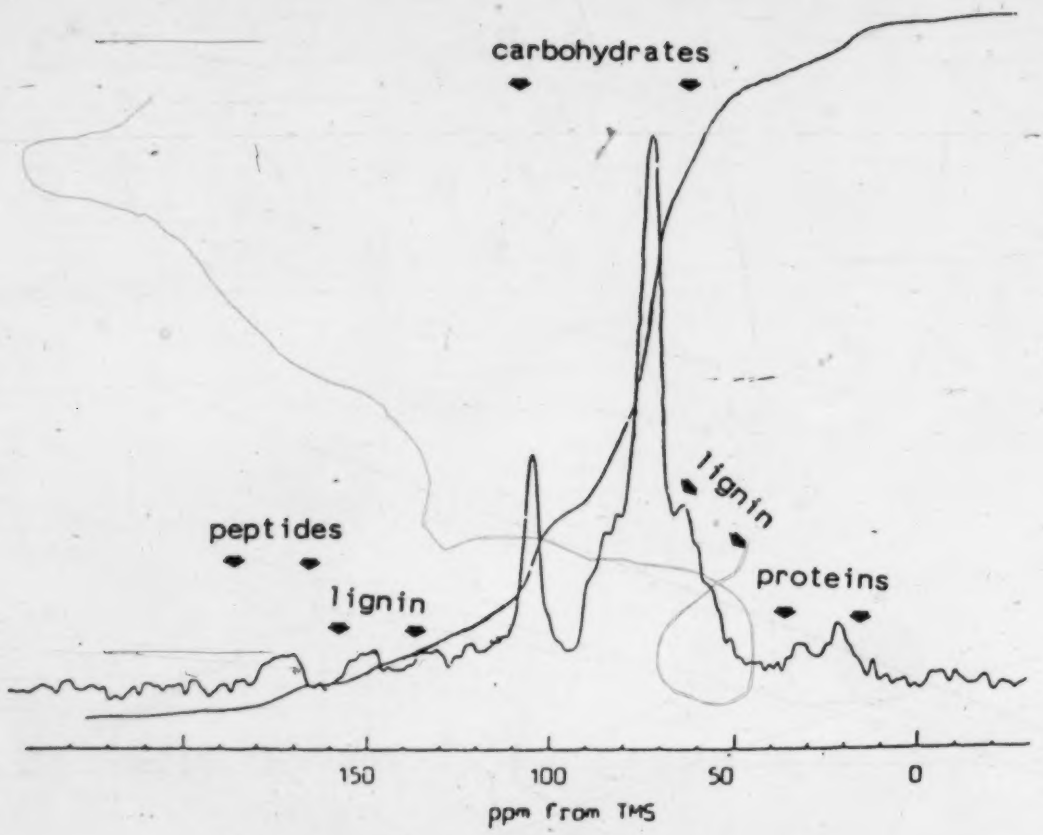

Fig. V.8. NMR spectrum of low buoyancy feces particles. The abcissa in this spectrum represents chemical shifts given in parts per million from the carbon of tetramethylsilane. This scale represents the magnetic shielding due to the electrons of chemically distinct carbon nuclei. On this scale, carbonyl carbons associated with peptide linkages, and with ester groups associated chiefly with hemicellulose appear at 170-180 ppm. The aromatic carbons, chiefly of lignin, appear at 130-160 ppm. Between 60 and 105 ppm oxygenated aliphatic carbon atoms associated chiefly with carbohydrates appear. The peak at 56 ppm is of the carbon of methoxyl groups, chiefly of lignin. At 5-50 ppm, aliphatic carbons appear that have been attributed largely to protein.

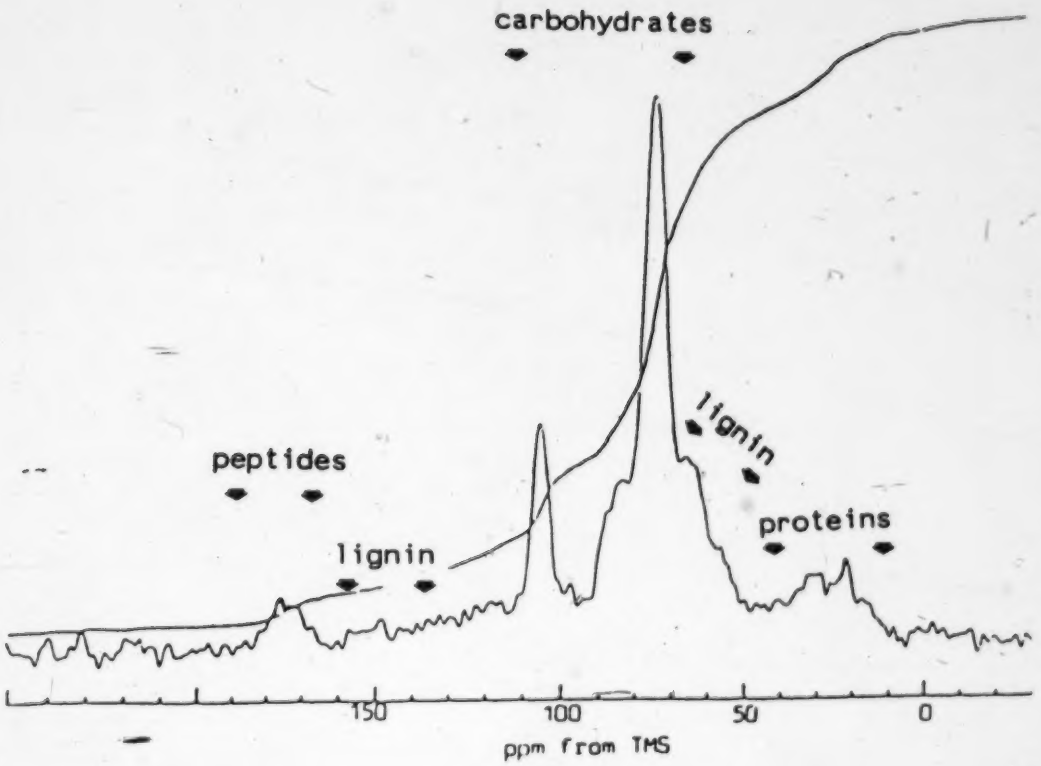

Fig. V.9. NMR spectrum of high buoyancy feces particles. The abcissa in this spectrum represents shifts given in parts per million from the carbon of tetramethylsilane. This scale represents the magnetic shielding due to the electrons of chemically distinct carbon nuclei. On this spectral scale, carbonyl carbons associated with peptide linkages, and with ester groups associated chiefly with hemicellulose appear at 170-180 ppm. The aromatic carbons, chiefly of lignin, appear at 130-160 ppm. Between 60 and 105 ppm oxygenated aliphatic carbon atoms associated chiefly with carbohydrates appear. The peak at 56 ppm is of the carbon of methoxyl groups, chiefly of lignin. At 5-50 ppm, aliphatic carbons appear that have been attributed largely to protein.

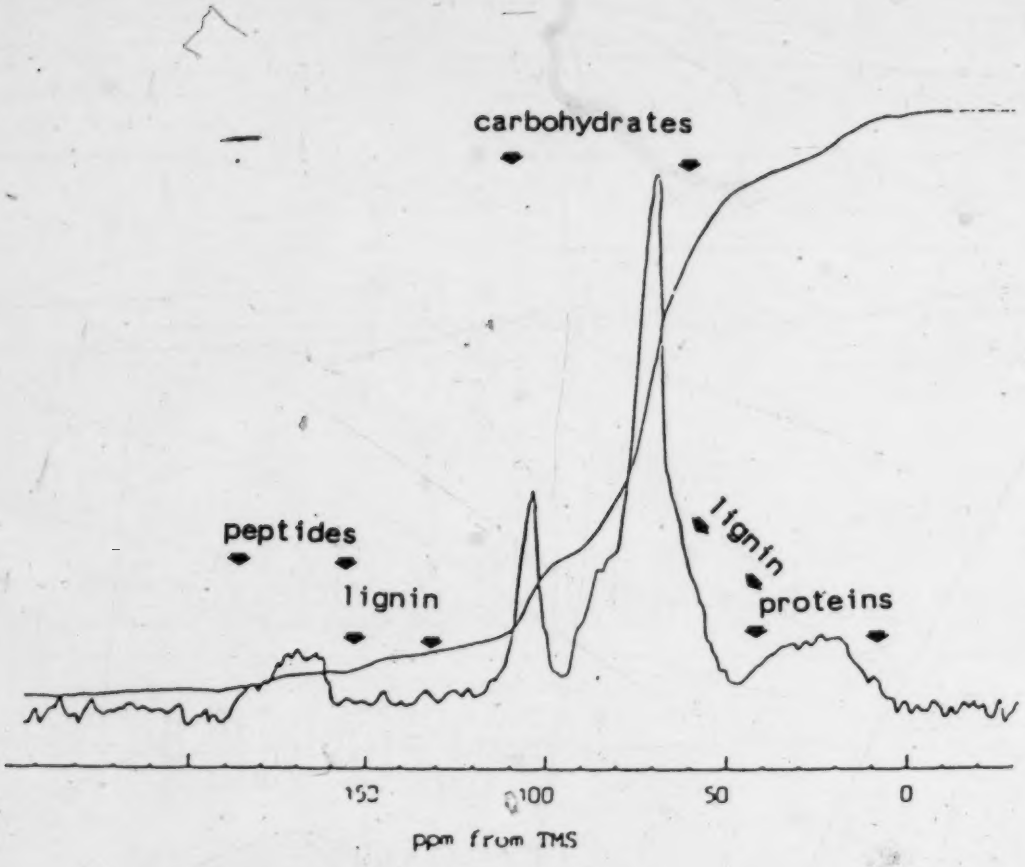

Fig. V.10. NMR spectrum of high buoyancy particles originating from the mat large particulate matter. The abcissa in this spectrum represents chemical shifts given in parts per million from the carbon of tetramethylsilane. This scale represents the magnetic shielding due to the electrons of chemically distinct carbon nuclei. On this spectral scale, carbonyl carbons associated with peptide linkages, and with ester groups associated chiefly with hemicellulose appear at 170-180 ppm. The aromatic carbons, chiefly of lignin, appear at 130-160 ppm. Between 60 and 105 ppm oxygenated aliphatic carbon atoms associated chiefly with carbohydrates appear. The peak at 56 ppm is of the carbon of methoxyl groups, chiefly of lignin. At 5-50 ppm, aliphatic carbons appear that have been attributed largely to protein.

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VI. EFFECTS OF PSYCHOLOGICAL STRESS, ACUTE COLD STRESS, AND DIFFERENT DIETS ON RETICULUM, RUMEN, AND OMASUM CONTRACTIONS IN CATTLE.

A. INTRODUCTION

Attempts to model rumen function (Ulyatt et al. 1976) have demonstrated a limited understanding of factors affecting rumen motility (Waghorn 1977). Evidence of relationships between motility and dietary factors has been reported (Colvin et al. 1958; Popescue and Florescu 1958; Freer et al. 1962). An understanding of these factors is important since any marked changes in the motility might be expected to affect the efficiency of digestion in the forestomach. However, most of the previous studies have involved a limited number of diets and have been conducted mainly in sheep (Colvin et al. 1958; Popescue and Florescu 1958; Reid 1963). Detailed studies of relationships between diet and gastric motility in cattle are needed because the anatomy and motility of their forestomachs differs from that of sheep (Reid 1963).

The summed excitatory and inhibitory effects of afferent inputs to the gastric motor centers play a major role in determining the pattern of motility exhibited by the reticulum and rumen at any given time (Stevens and Sellers 1959; Comline and Titchen 1961). Gastric motor

centers are affected not only by afferent inputs from the gut but also by the activity of higher centers in the brain which may be influenced by psychological stress. In addition, nerve and muscle cells are sensitive to changes in the composition of the extracellular fluids that bathe them. Changes in the composition of tissue fluids may occur as a result of the digestion of food in the gut or hormonal changes. At present, there is little information on the extent to which tissue fluid changes may affect gastric motility (Reid 1963).

Sensory inputs arising from environmental stimuli, such as temperature, may also influence the gastric centres. Cold exposure has been observed to increase frequency of reticulum contraction in sheep (Westra and Christopherson 1976) and in cattle (Gonyou et al. 1979) However, these studies were done after many hours of exposure to the cold environment. Little information is available regarding the effects of acute cold stress on gut motility of steers and its interaction with different types of diets.

The objectives of the present experiment were to determine the effects of different types of diet on reticulum, rumen, and omasum contraction frequencies in steers and to assess the influence of a psychological stress and acute cold stress on the ruminant stomach movements. Finally, the interractions between diet and type

of stress on stomach motility were investigated.

MATERIALS AND METHODS

Animals and their Management

Four 15-month old Hereford steers, each fitted with a rumen cannula (10 cm diameter) were maintained in individual pens (3 m X 4 m) at an ambient temperature of 21 ± 1^{0}C. Four diets were offered to each steer, in a Latin Square design. The diets included Bromegrass (Bromis inermis) hay - B, Alfalfa (Medicago sativa) hay - A, Alfalfa silage - AS, and a barley diet (Hordeum vulgare) - C. The daily intakes were calculated to meet the maintenance requirements of the steers (NRC 1984, Table 1). All animals were fed by means of an automatic feeding device which delivered the daily ration in 12 equal portions every 2 hours. The steers were adapted to their respective diets for at least 3 weeks before the recording periods started. The B, A, and AS diets were chopped through a 76 mm screen and were harvested at the mid-to late-bloom stage. Components of each diet are shown in Table VI.1 . Water and Co Iodized salt were available ad libitum.

Motility measurements

Prior to experiments, the steers were placed in a squeeze to restrict movement and to facilitate access to the rumen cannula. Motility of the reticulum, ventral sac of the rumen, and the omasum were monitored using open

ended catheters (Polyvinlychloride, 0.09 mm i.d.) inserted to each region. The catheters were coupled to pressure transducers (Gould Statham). A constant flow (75 mL h^{-1}) of water was maintained in the catheters using a continuous infusion pump (Ismatec MT-13). Changes in pressure in each region were recorded using a Beckman (model R612) recorder. The recording started at 0800 h and was done in such way that feeding times occured before and after the recording times. The animals were used always in the same chronological order during each recording period. The animals were not fed when they were exposed acutely to the cold environment (for 4 h). However, the recording period was between two normal feeding times (between the third and fouth hour).

Diet Effects

During the first recording day, effects of intake of the diets in the control environment on the motility of the forestomachs were measured without any additional stress being imposed on the animals. Recording of the contractions of the various areas in the gut continued for a minimum of 1.5 h.

Psychological Stress

On the second day of recording, to add a psychological stress to the cattle, a trained German Shepherd dog was positioned 1 m in front of the steer's head for a period of 10 - 15 minutes, thereby providing visual, auditory, and

olfactory stimuli. The dog was trained to sniff, growl and bark, but was not allowed to touch the steer. Motility was measured before exposure to the dog (.75h), as well as during (.25h) and after (.75h) removal of the dog.

Acute Cold Stress

On the third day, animals were placed in stanchions in a cold chamber maintained at a constant temperature of $-20^{0}C$ for a minimum of 3 hours prior to measurement of gut motility. Three hours results in an acute cold stress (B. A. Young, personal communication). Recordings of motility were continued for 1 hour after the 3 h period. During this period, animals were not allowed access to feed or water.

Measurement of Heart Rate

E.C.G. surface electrodes were placed on the animal. Heart rate was recorded during all trials from the E.C.G. Beckman recorder adaptor (model 9857).

Statistical Analysis

The time between each rumen, reticulum and omasum contraction was measured individually and the average number of contractions per hour calculated for each recording period. The data were analyzed by analysis of variance using a Latin square (4 X 4) design with four steers (columns), four recording periods (rows) and four diets (treatments). For comparisons, the model was a Latin square with a repeated measure. The interactions between the diets and the effects of the psychological stress, as

well as the acute cold stress effects on the ruminant stomach movements were calculated. The means were compared by Student-Newman-Keuls' test (Steel and Torrie 1980).

C. RESULTS

The mean motility values are presented in Table VI.2 for control, acute cold stress and during psychological stress. In general, diets did not influence reticulum, omasum and rumen contraction frequencies with the exception of the rumen in acute cold stress ($p<0.01$) and omasum during psychological stress ($p\leq0.04$). Moreover, dietary differences did not influence the E.C.G. frequency.

The contraction frequencies of the omasum and rumen were increased ($p<0.05$ and $p<0.01$, respectively) by acute cold stress (Table VI.3) but the increase in reticulum movement frequency was not significant ($p>0.05$). There was no significant ration/stress interaction. Heart rate was increased ($50 \pm 5\%$, $p<0.01$) by acute cold stress (Table VI.3).

Statistical analysis of the results for the four periods (pre-, during, post- and post 20 min) of psychological stress study indicated that the contraction frequency of the forestomachs was increased ($p<0.01$) during the presence of the dog. The contractions during the pre-, post- and post 20 min stress periods were not different. There was no significant interaction between the rations

and the psychological stress. The E.C.G. frequency was increased (80 ± 5%, $p<0.01$) by the psychological treatment (Table VI.4).

Reticulum contraction frequency was increased to a greater extent by psychological stress than by acute cold stress. Omasum and rumen frequencies during psychological stress and acute cold stress did not differ. The rations did not interact significantly with the comparison between the two stresses (Table VI.5).

Correlations between E.C.G. and movements of the forestomach when all treatments were pooled indicated a high and significant ($p<0.01$) R^2 value for the reticular contractions in three out of four steers and for the omasal contractions for two out of four steers. However, there was no significant relation between heart rate and rumen contraction frequencies in any of the steers (Table VI.6). The correlations between the above parameters when the steers were in pre psychological stress and control trials were not significant ($p>0.05$, Table VI.7). The T test done on the pooled values from the four steers indicated that the heart rate explained a large part of the variation of the forestomach contraction frequencies when all treatments were pooled ($p<0.001$, Table VI.6) and when variation between steers has been removed. However, there was no significant T value when the animals were pooled in pre-psychological stress and control trials (table VI.7).

D. DISCUSSION

DIETS

Our observation that the different diets had, in general, no significant effect on the frequency of the reticulum, omasum and rumen contractions is in agreement with Waghorn and Reid (1977). These researchers found that in radiographic studies involving sheep fed three diets (pelleted lucerne, chaffed lucerne hay and Ruanui ryegrass) the patterns of digesta movement were similar in all sheep and showed little variation between the three feeds. In addition, rumen motility was similar for the three diets.

However, motility of the forestomachs appears to be related to variations in condition of animal and change in some dietary factors. Relationships between the motility of the forestomachs and fasting, feeding, or ruminating states have been demonstrated (Nesic 1960; Phillipson and Reid 1960). Evidence of relationships between the motility and dietary factors has also been reported in other studies (Colvin et al. 1958 ; Popescu and Florescu 1958; Freer et al. 1962). However, the extent to which motility changes with different circumstances has not been thoroughly investigated.

The extent and persistence of the changes in the motility of the reticulum during feeding appear to be influenced by dietary factors (Reid 1963). These

include the amount of the feed eaten during the present meal, the interval of time elapsed since the last meal, and the amount of the feed eaten during that and earlier meals. For a given feed and an individual animal, there is a direct relationship between the amount eaten and both the extent and persistence of the changes in form of the movement sequences during feeding (Reid 1963).

The mean daily frequency of rumino-reticulum contraction rises with increased intake and is related to more time spent eating and ruminating. There is a direct relationship between the intake of roughage and the mean frequency of reticular contraction while cattle are resting (Waghorn and Reid 1977). The relationship with diets is altered when the time of access to a roughage is sufficiently restricted to cause an appreciable rise in the rate of eating.

In the present experiment, the animals were not fed during the recording period, being fed at 1/12 maintenance diet every 2 h such that the feeding times occurred before and after the recording time. Detection of variation in forestomach motilities expected as a consequence of diet intake and fasting and feeding factors was therefore minimized in this experiment. This approach might explain why we did not find an effect of diet on motility of the forestomachs. Our observations, as well as previous papers (Stevens et al. 1960; Reid 1963), indicate that the relationship between the diet and the motility of the

rumino-reticulum depends on many factors.

Acute cold stress

Our observation of a cold stress - induced increase ($p<0.06$) in rumen and omasum contraction frequency, but a non significant ($p<0.14$) increase in reticulum motility during cold exposure differ only slightly from the results of Westra and Christopherson (1976), Gonyou et al. (1979) and Kelly and Christopherson (1985) who reported an increase for the reticulum contraction frequency in the cold. However, our results have extended the observations to include a more detailed analysis of motility responses of the rumen and omasal compartments in addition to reticulum motility in animals exposed to acute cold stress.

Contractions of the forestomachs are presumed to be important in regulating the flow of material from the rumen to the abomasum (Titchen 1968). Increased forestomach motility during cold exposure would be expected to enhance the rate of passage of small particles from the rumen by promoting their mixing, sorting and fluid propulsion (Christopherson and Kennedy 1983).

The fact that the increased contraction frequency of the rumen and omasum occurred after only 3-4 h of cold exposure indicated the rapidity of this reaction. This is in agreement with observations on reticulum motility in sheep reported by Westra and Christopherson (1976), and in cattle reported by Gonyou et al. (1979). The rapidity of

the motility responses suggests that a neural mechanism may be involved (Christopherson and Kennedy 1983).

The fact that we did not feed the animals during the acute cold exposure does not invalidate the trial since the contraction frequency of the forestomachs normally decreases after feeding. Indeed, Dracy et al. (1972) found the average duration of contraction cycles to be 37.3, 43.8 and 56.5 seconds while eating, resting and ruminating, respectively. In spite of this normal decrease between feeding periods we recorded an increase of the contraction frequencies of the forestomachs during acute cold exposure.

Interaction between diets and cold stress

Our finding of no interaction between diets and acute cold stress effects on motility is in agreement with the following experiments. Although Warren et al. (1974) studied the effect of temperature on the digestion of three different forages (alfalfa, orchardgrass and tall fescue) they did not report any interaction between diet and environment. The results of Kennedy (1985) similary do not support a hypothesis that the effect of cold exposure on digestion is dependent on the physical form of the diet at fixed intake. Kennedy (1985) indicated that increased voluntary feed intake in the cold was a result of increased clearance of digesta from the rumen through decreased rumen particle size and increased reticulum motility.

E.C.G. and acute cold stress

Our observation of an increased heart rate in acute cold stress indicates a rapid state of stress defence by the animals. This is in agreement with Westra and Christopherson (1976) who stated that the reduced retention time and digestibility in a cold environment might be due to increased thyroid activity with cold exposure. Graham et al. (1981) indicated that the sympatho-adrenal medullary activity in ruminants is increased in cold environment. A rapid increase of thyroid hormones (T_3, T_4) and adrenergic hormones would likely significantly increase the heart rate (Guyton 1981).

Psychological stress

Acute psychological stress is likely to activate the adrenergic nervous system as well as adrenal medullary activity. Indirect evidence for this in the present experiment was provided by the significant increase of the steer heart rates in presence of the barking dog. One might expect acute psychological stress to be associated with decreased rather than increased motility of the forestomach since catecholamines usually inhibit gastrointestinal nonsphincter contractility (Guyton 1976). This also seems to be the case in the unanaesthetized intact ruminant (reviewed by Habel 1956). However, Titchen (1968) reviewed various studies in which injections of adrenalin or

stimulation of sympathetic nerves induced rumino-reticulum contractions. Ali et al. (1976) reported that intravenous adrenalin injections (1-2 ug kg^{-1}) induced either a single contraction or series of contractions of the rumino-reticulum during periods in which motility was inhibited by insulin-induced hypoglycemia. Dussardier (1954) reported that adrenalin treatment facilitated the reflex contractions of the reticulum induced by afferent stimulation of a vagus nerve. Ruckebusch (1971) reported that adrenalin injections (2 ug kg^{-1}) had a brief stimulating effect followed by inhibition of all stomach compartments in sheep and cattle. Graham et al. (1982) found that a higher dose of adrenalin was required to inhibit rumen motility in cold-acclimated compared to warm-acclimated sheep and that rumen motility tended to be enhanced in cold-acclimated sheep but inhibited in warm-acclimated sheep, during noradrenalin infusion. Christopherson and Kennedy (1983) suggested that catecholamines may cause facilitation of reflex responses to sensory stimuli (such as distension) that normally increases motility and at the same time they may have some direct inhibitory effects on the gastrointestinal musculature. Stresses (psychological or cold stress) may alter a balance between facilitation of reflex activation and direct inhibition of gut musculature.

The reticulum seems to be more affected by the psychological stress than the cold stress. The rumen and

omasum contractions were similarly affected by the two stresses. The diets did not influence the effect of the two stresses.

Correlations between heart rates and forestomach movements

The high correlations between the pooled heart rate and the reticulum and omasum movement frequencies indicated that when the reticulum and omasum contractions increased the heart rate increased as well. This indicated that the contraction frequencies of these two forestomachs and the heart accelerated during stress. However, there was no correlation between heart rate and forestomach contractions during the control situations. Thus stresses (acute psychological and acute cold stress) which activate the sympathetic nervous system and the sympatho-adrenal medullary complex have qualitatively similar effects on the heart and the forestomach contraction frequencies.

The rumen contraction frequency, when the treatments were pooled, did not have as a high correlation with the heart rate as the reticulum and omasum for individual animals. This is probably due to the B sequence contractions which are related mainly to the rate of gas formation in the rumen and the process of eructation. This gas production may not be correlated with the activity of the sympathetic nervous system.

Conclusions

The conclusions reached from this experiment are the following:

1: The nature of the diet, when given at maintenance level and divided in 12 identical feeding periods during a day, did not influence the frequency of forestomach contractions during the non - feeding periods.

2: Type of diet did not interact with the effects of acute cold stress or acute psychological stress on forestomach contraction frequencies.

3: Acute cold stress as well as the acute psychological stress significantly increased the contraction frequency of rumen and omasum.

4: Psychological stress was more effective than the acute cold stress in stimulating reticular contractions.

TABLE VI.1: CONSTITUENTS OF THE DIETARY DRY MATTER

ITEMS[@]	BROME HAY	ALFALFA HAY	ALFALFA SILAGE	BARLEY GRAIN
DM (%)	90.12	94.08	75.13	89.93
CP (%)	11.54	15.76	14.18	9.12
ADF (%)	32.70	33.65	26.71	6.92
MAINTENANCE INTAKES (Kg d^{-1})	5.84	5.55	6.96	3.34

[@]DM= dry matter; CP= crude protein; ADF= acid detergent fiber.

TABLE VI.2: EFFECTS OF DIETS ON RETICULAR, OMASAL, RUMINAL MOVEMENTS (contractions h^{-1}) AND HEART RATE (contractions min^{-1}) IN CONTROL ENVIRONMENT, ACUTE COLD AND PSYCHOLOGICAL STRESS.

ORGANS	DIETS 1 concentrate	2 alfalfa	3 brome hay	4 silage	S.E.M.
CONTROL					
Reticulum	69.5	70.5	73.1	66.5	3.2
Omasum	67.1	70.9	97.7	78.8	8.6
Rumen	137.9	118.5	92.0	113.5	25.1
Heart rate	40.8	50.2	47.5	45.8	1.3
ACUTE COLD STRESS					
Reticulum	75.2	84.5	72.9	73.7	8.2
Omasum	119.1	88.3	110.4	98.9	14.8
Rumen	149.8	224.4	128.7	235.3	14.9**
Heart rate	72.7	88.4	61.0	57.8	8.0
PSYCHOLOGICAL STRESS					
Reticulum	125.2	101.0	82.3	106.7	23.2
Omasum	118.8	88.5	97.5	134.6	18.9*
Rumen	199.9	185.3	138.2	173.9	20.3
Heart rate	61.4	81.8	80.1	95.4	6.4

* $p<0.05$
** $p<0.01$

TABLE VI.3: EFFECTS OF ACUTE COLD STRESS ON THE CONTRACTION FREQUENCIES (CONTRACTIONS h^{-1}) OF THE RETICULUM, RUMEN AND OMASUM AND HEART RATE (CONTRACTIONS min^{-1}) IN STEERS.

	TREATMENTS				
ORGAN	CONTROL	COLD	S.E.M.	SIG OF F	RATIONS*STRESS SIG.[e]
-RETICULUM	69.93	76.58	2.75	0.14	0.66
-OMASUM	78.63	105.23	7.17	0.05	0.56
-RUMEN	115.47	184.54	11.15	0.005	0.13
-HEART RATE	46.09	69.95	3.28	0.02	0.37

[e]Significance of interaction between diet factor and acute cold stress factor on organ contraction frequency.

TABLE VI.4: EFFECTS OF ACUTE PSYCHOLOGICAL STRESS ON THE CONTRACTION FREQUENCIES OF THE RETICULUM, RUMEN, OMASUM (CONTRACTIONS h^{-1}) AND HEART RATE (CONTRACTIONS min^{-1}).

ORGANS	PRE.	DURING	POST	POST 20min.	S.E.M.	RATION by PSY.STR. SIG@.
RETICULUM	66.95a	103.80b	72.96a	67.22a	6.21**	0.70
OMASUM	71.01a	109.83b	77.39a	71.87a	4.94***	0.38
RUMEN	113.17a	168.96b	127.24a	108.83a	7.52***	0.28
HEART RATE	44.06a	79.66b	46.42a	46.34a	2.47***	0.22

a, b: Values in the same row followed by the same letter are not significantly different ($p<0.05$). (Student-Newman-Keuls' test).

* $p<0.05$

** $p<0.01$

*** $p<0.001$

@Significance of interaction between diet factor and psychological stress factor on organ contraction frequency.

TABLE VI.5: COMPARISON BETWEEN THE CONTRACTION FREQUENCIES OF THE FORESTOMACHS (CONTACTIONS h^{-1}) AND HEART RATE (CONTRACTIONS min^{-1}) DURING PSYCHOLOGICAL AND COLD STRESS.

ORGANS	STRESS		S.E.M.	SIGNIFICANCE OF F	RATION*STRESS[1] SIGNIFICANCE
	PSYCH.	COLD			
RETICULUM	103.8	76.6	7.3	0.04	0.55
OMASUM	109.8	105.2	8.5	0.66	0.57
RUMEN	168.9	184.5	11.2	0.36	0.09
HEART RATE	79.7	69.9	3.3	0.14	0.09

[1]Interaction between diet factor and stress factor on organ contraction frequency.

TABLE VI.6: CORRELATIONS BETWEEN HEART RATE AND FORESTOMACH MOVEMENTS WHEN ALL TREATMENTS ARE POOLED.

STEER	ORGANS	R^2	SIGNIFICANCE[1] OF R^2
ONE	reticulum	0.464	0.001
	omasum	0.404	0.002
	rumen	0.231	0.022
TWO	reticulum	0.528	0.000
	omasum	0.034	0.233
	rumen	0.085	0.120
THREE	reticulum	0.718	0.000
	omasum	0.726	0.000
	rumen	0.283	0.016
FOUR	reticulum	0.137	0.065
	omasum	0.059	0.164
	rumen	0.179	0.040

ANIMALS POOLED	COMPARTMENT	T TEST[2] VALUE	T VALUE SIGNIFICANCE
	reticulum	4.349	0.000
	omasum	3.801	0.000
	rumen	3.682	0.000

[1]Test of the null hypothesis: probability to be wrong if we claim that the correlation is real.
[2]T TEST: Indicates how much variation of the forestomach contraction frequencies the heart rate explains when variation of steers has been removed.

TABLE VI.7: CORRELATIONS BETWEEN HEART RATE AND MOVEMENTS OF THE FORESTOMACH WHEN THE STEERS ARE NOT UNDER THE ACTION OF STRESS.
(PRE-PSY. & CONTROL POOLED)

STEERS	COMPARTMENT	R^2	SIGNIFICANCE[1] OF R^2
ONE	reticulum	0.197	0.189
	omasum	0.137	0.235
	rumen	0.393	0.092
TWO	reticulum	0.852	0.004
	omasum	0.211	0.180
	rumen	0.562	0.043
THREE	reticulum	0.022	0.389
	omasum	0.014	0.411
	rumen	0.278	0.141
FOUR	reticulum	0.331	0.116
	omasum	0.029	0.374
	rumen	0.469	0.066

ANIMALS POOLED	COMPARTMENT	T TEST[2] VALUE	SIGNIFICANCE OF T VALUE
	reticulum	1.875	0.076
	omasum	0.624	0.540
	rumen	0.930	0.364

[1] Test of null hypothesis; probability to be wrong, if we claim that the correlation is real.
[2] T TEST: Indicates how much variation of the forestomach contraction frequencies the heart rate explains when variation of steers has been removed.

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VII. GENERAL CONCLUSIONS

The first experiment reported herein indicated that the rumino-reticulum is the principle site of the digestion of feed particulate DM. This is in agreement with authors such as Hunter and Sieberg (1980), Redman et al. (1980), Kennedy (1982) and Srikandarajah et al. (1982). The large particle pool appeared to be very important for the production of VFA and CO_2. A substantial portion (20 $\pm$ 11%) of the hay was rapidly solubilized. This is supported by Ulyatt et al. (1986) who stated that 20-30% of the diet DM is solubilized by chewing with dried diets. The kinetic technique indicated that the mass ratio of the large particle pool (>3.35 mm) to the small particle pool (<3.35 mm) in the rumino-reticulum was 2:1. This agreed with wet sieving technique. The small and large particle pools were reasonably represented as homogenous first order kinetic pools. This is in agreement with experiments (Dixon and Milligan 1985) in which disappearance from the rumen of fluid, particulate,and microbial markers, occurs according to first order kinetics. The present study indicated that low level Cr mordanting of neutral detergent-extracted forage particles is a good technique to attain biologically realistic measurement of particle kinetics in the rumino-reticulum.

The second study showed a high degree of coincidence between inspiratory movements and the second biphasic

contraction of the reticulum. This may favor passage of digesta to the omasum. In view of our results, a synaptic link between respiratory centers and the rhythmic command center which control the motor activity of the ruminant forestomach appears possible.

The third study determinated that the present fiber-optics endoscopic technique is useful in visualizing the kinetic characteristics of the particulate rumino-reticulum phase. The pattern of movement of the small and large particles was relatively similar. The general movement appears to be a relatively simple circular stream inside the rumino-reticulum. The present study agrees partially with results of Waghorn and Reid (1977) who used high resolution X-Rays.

The fourth study indicated that there is an interrelationship between buoyancy, physical attributes, shape, size, chemical and nutritive composition of different types of particles. This is supported by Van Soest (1982) who stated that nutritive value of forages is greatly affected by physical form and other physical attributes, and that the shape and nature of particles are related to their chemical composition. Ehle and Stern (1984) stated that the distinction between chemical and physical characteristics of feedstuffs is usually vague, since chemical composition often ultimately determines the physical attributes of feed (e.g. density and particulate shape). Relative buoyancy might be useful in an indirect

analysis of the nutritive value of forage particles.

In the last experiment the combination of diets and the methodology did not produce a significant modification on the contraction frequencies of the forestomachs and did not interact in general with the effect of the stresses. Acute cold stress and psychological stress produced significant increases in the contraction frequencies of the forestomachs. Stresses may alter a balance between facilitation of reflex activation and direct inhibition of gut musculature (Christopherson and Kennedy 1983). Our finding of no interraction between the effect of different diets and acute cold stress on forestomach motility is in agreement with Warren et al. (1974) and Kennedy (1985).

A better understanding of the forestomach movements and digesta kinetics in ruminants has been achieved by the present studies.

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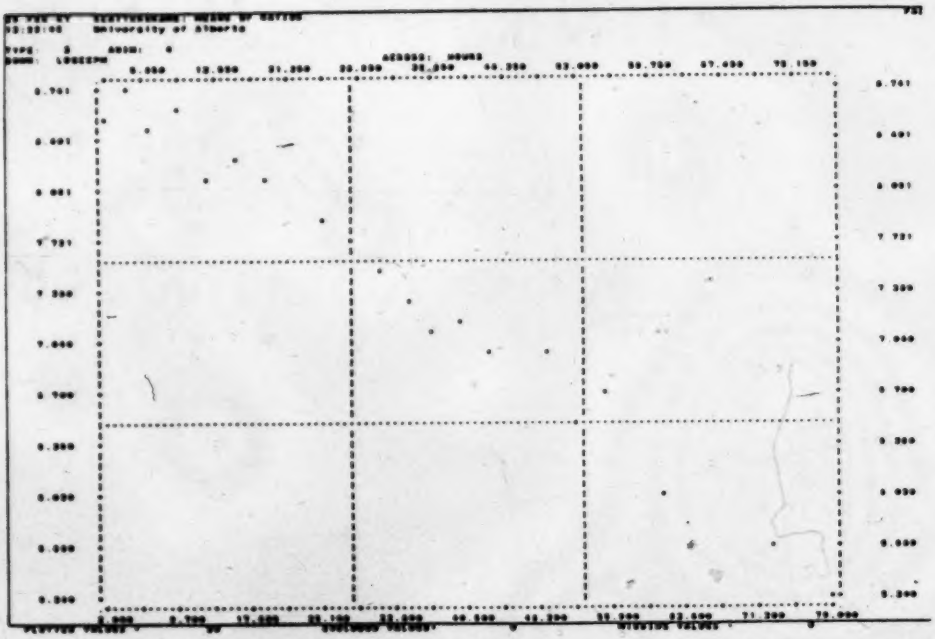
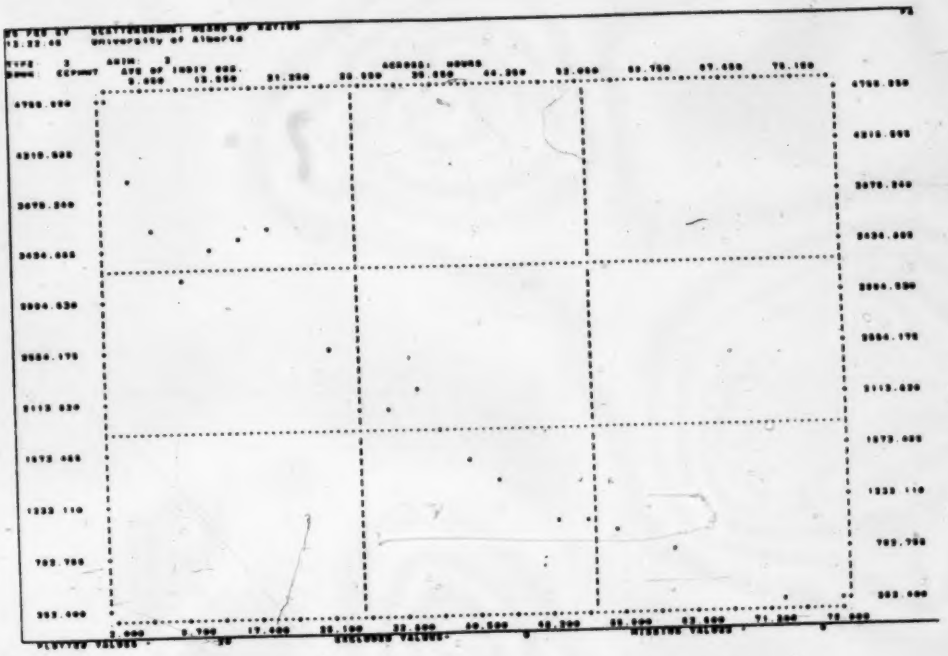
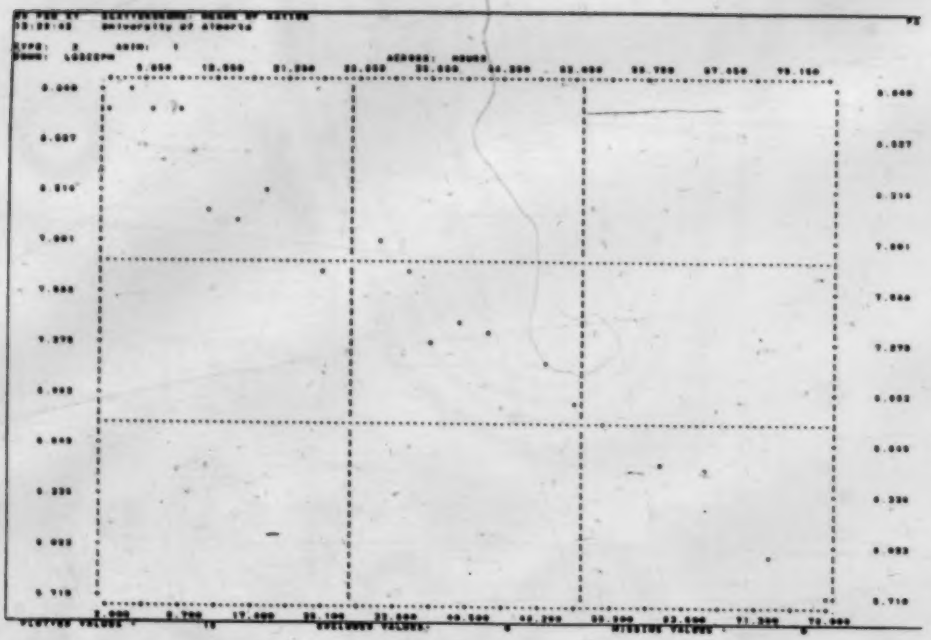
Appendix 1

Examples of disappearance curves from rumen sampling of NDF brome stems mordanted with low concentration of Cr when the count per minute is transformed to logarithmic base.

- Example 1: Animal 4, small particles (0.01% Cr).



Examples 2: Animal 3, large particles (0.01% Cr).



Examples 3: Animal 1, small particles (0.01% Cr).